SOMATIC CROSSING OVER AND SEGREGATION IN DROSOPHILA MELANOGASTER*

CURT STERN
University of Rochester, Rochester, N. Y.

Received May 6, 1936

TABLE OF CONTENTS

* A part of the cost of the accompanying tables and figures is paid by the Galton and Mendel Memorial Fund.

INTRODUCTION

IN 1925 BRIDGES found that females of *Drosophila melanogaster* containing the dominant factor Minute-n in one X chromosome and some recessive genes in the other X chromosome often exhibit a mosaic condition. While the main surface area of these flies showed the effect of the dominant Minute-n (I, 62.7) without the effect of the recessive genes, as was to be expected, smaller areas, in different regions of the body and of varying size, were not Minute-n, phenotypically, but displayed the effects of the recessive genes. BRIDGES' interpretation was this: the Minute-n factor has the property to eliminate occasionally the X chromosome in which it itself is located. The cells of mosaic spots are descended from one common ancestral cell in which such elimination had taken place. They possess, therefore, only one X chromosome and show the phenotype produced by its genes.

Minute-n is only one of a group of factors which are very similar in their phenotypical expression. The "Minutes" behave as dominants whose most striking phenotypic effect is a reduction in bristle size; in addition there is a strong retardation in development, tendency to rough eyes, etc. The homozygous Minute condition is lethal. Some Minutes have been shown to be deficiencies (SCHULTZ 1929). Many Minute factors have been found in different loci of all chromosomes. They are distinguished by adding different letters or numbers to the symbol *M*.

Following BRIDGES' discovery of mosaics with respect to sex-linked factors, the appearance of mosaic spots which exhibit autosomal characters was described (STERN 1927b). Such spots appear on flies which originally had a constitution heterozygous for genes determining the characters. These mosaics occurred in crosses in which autosomal Minute factors were present and the facts seemed to agree with the interpretation that the spots were due to an elimination of that arm or part of an autosomal chromosome which carried the Minute.

The present investigation was originally designed to attack the problem: How is a Minute factor able to eliminate the chromosome or that part of a chromosome in which it itself is located?

At the same time the solution of another problem was sought. The fact that small mosaic spots showed the phenotypic effect of certain genes contained in their cells whereas the remainder of the individual showed another phenotype was proof of the autonomous development of these char-

acteristics. Among the very few genes which did not show phenotypical effects in spots was the recessive "bobbed" (I, 66.0) which produces short bristles: in $+^{v}Mn+^{bb}/y+^{Mn}bb$ females the $y+^{M}$ spots did not possess the *bb*-type bristle length, but a $+^{bb}$ length. Non-autonomous development of the bobbed character seemed improbable as typical gynandromorphs had shown clear demarcation lines for the *bb* and $+^{bb}$ areas (STERN 1927a). As bobbed is located at the extreme right end of the genetic X chromosome, next to the spindle fibre attachment, the following hypothesis was proposed: just as in the case of autosomal eliminations only part of the autosome disappears, so also in *Mn* mosaics merely a portion of the X chromosome is eliminated. The piece adjoining the spindle attachment and including the bobbed locus is assumed to be left in the cell, thus giving a constitution $+^{bb}/y+^{Mn}bb$, which, being heterozygous for *bb*, does not produce the effect of this gene (STERN 1928b). When PATTERSON (1930) using MULLER'S Theta translocation showed that in his cases of X-radiated flies "not the whole X chromosome was eliminated" it was decided to use the same genetic technique to test the above hypothesis as to the partial elimination in the case of *Mn*. The Theta translocation was kindly put at my disposal by Prof. H. J. MULLER.

Both problems, the question as to the action of Minutes to bring about elimination and the question as to complete or partial elimination of the X chromosome (intimately bound up with the question as to autonomous or non-autonomous development of bobbed in small spots), proved to be based on an erroneous concept as to the origin of mosaic spots. While the investigation revealed this, it provided at least a partial solution of the problems by the discovery of somatic crossing over and segregation in Drosophila.

ACKNOWLEDGMENTS

Some of the work with autosomal Minutes was carried out in 1926 at the Department of Zoology of Columbia University in New York City under a Fellowship from the International Education Board. These experiments were continued at the Kaiser Wilhelm Institut für Biologie in Berlin-Dahlem. The greater part of the investigation proceeded since December 1932 in the following institutions: Kerckhoff Biological Laboratories, California Institute of Technology, Pasadena, California (Fellow of the Rockefeller Foundation); Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. (guest); and Department of Zoology, University of Rochester, Rochester, N. Y. The author is grateful for all the help given him everywhere, especially for the very generous hospitality at Cold Spring Harbor extended to him in a difficult time by Prof. C. B. DAVENPORT and Dr. M. DEMEREC.

For suggestions concerning the manuscript thanks are due to members of the Biological Laboratories in Pasadena and to my wife, EVELYN STERN.

METHODS

The methods were similar to those used by BRIDGES. Flies were made heterozygous for recessive genes whose phenotypic effects were of such a kind as to be exhibited by very small areas, preferably even single setae. The setae of Drosophila are divided into macrochaetae and microchaetae, the former generally called bristles, the latter, hairs. As far as the purpose of the present study is concerned the distinction is of no intrinsic importance. Genes mainly used were: (a) yellow body-color (*y*, 1, 0.0), producing an effect which can be distinguished in a single hair, making it yellowish-brownish as opposed to the black not-yellow condition (the general coloring effect of *y* on the hypodermis is often not very distinct in spots (STURTEVANT 1932)) and (b) singed-3 (sn^3, 1, 21.0), producing a thickened, curved or crooked condition of the setae, which generally can be distinguished in single hairs also. However, doubts occasionally remain as to whether a single hair on a heterozygous $+/sn^3$ fly is genotypically singed or whether it is normal but slightly more bent than usual. With spots of two or more hairs such doubts hardly ever occur.

Following BRIDGES, the flies were inspected originally for spots only on the head and thorax. In later experiments, however, inspection of the abdomen was included. In order to discover even the smallest spots the flies were scrutinized under a binocular magnification of 37× (Bausch & Lomb objective 3.7, eyepieces 10×). The use of a simple device made by the Bausch & Lomb Optical Company which allows for the fine adjustment to be made by foot movements and leaves both hands free for manipulation, proved to be of great value (for more detailed description see Drosophila Information Service 6:60).

As the study of spots was practically confined to setae-bearing regions, a table of the number of setae on different parts of the body of average sized females was computed. Generally only the dorsal and lateral parts of the thorax and only the tergites of the abdomen were inspected and, except in special cases, no effort was made to remove the wings in order to uncover completely the median part of the abdominal tergites. This partly covered region excluded about 20 per cent of the abdominal setae from inspection (table 1).

A separate record was kept for each spot consisting of an outline drawing in case of head and thorax mosaics or of a notation of number of macro- and microchaetae and position in case of abdominal mosaics.

Spots on the dorsal side of the thorax nearly always form one single clearly defined mosaic area, while spots on abdominal tergites are fre-

TABLE 1

Number of setae on different body regions.

	HEAD*	THORAX*	ABDOMINAL TERGITE					
			1 AND 2	3	4	5	6	7
Mean number of setae	34	238	108	120	117	123	120	61
Not inspected (estimate) %	0	0	50	40	20	0	0	0
Inspected	34	238	54	72	94	123	120	61
% of total inspected setae	4	30			66			
Inspected abdominal setae (% of total)			10	13	17	24	24	12

Total setae inspected: ca. 800.
Total abdominal setae inspected: ca. 525.
* Dorsal parts only.

quently broken up into two or more separate parts. Apparently the growth processes in the imaginal discs of thorax and abdomen are somewhat different.

THE ACTION OF MINUTE FACTORS

Blond-Minute

As stated in the introduction, a number of Minute determiners have been found to be deficiencies for short regions. The hypothesis suggested itself that the apparent tendency of Minutes to eliminate the chromosomes or chromosomal parts in which they are located is due to some mechanical disturbance of chromosome division which itself is caused by the material defect in the deficiency chromosome.

In order to test this hypothesis use was made of the "Blond-translocation" (Bld) which represents a reciprocal translocation between the left end of the X chromosome and the right end of the second chromosome (BURKART and STERN 1932). When all chromosomes of an individual are balanced with respect to the translocation, normal sized Blond bristles are produced. However, in females if one X chromosome lacks its extreme left end without being compensated for by the presence of the translocated piece on the second chromosome, then the individuals possess bristles of Minute, Blond character. BURKART had found that these Minute females

show mosaic spots, a fact which seemed to indicate that elimination of the deficient X chromosome occurred.

The viability of Blond-Minutes is rather low and depends greatly on culture conditions. In half-pint milk bottles with one pair of parents their viability was found to average 30 per cent of their not-Minute sisters. Under PATTERSON'S culture conditions the Minutes apparently never appeared and this led him to assume a viability factor in the region covered by the deficiency (PATTERSON 1932). The absence of this factor was believed to cause the death of the zygote.

In a Blond male the left end of the X is translocated to the right end of one of the second chromosomes. Such a male forms two kinds of X carrying gametes: (1) deficiency X, translocation II; (2) deficiency X, normal II. Mated to a normal female, two kinds of daughters are produced: (1) def./normal; transl. II/normal II; (2) def./normal; normal II/normal II. The females (1) are not-Minute, the females (2) are Minute. *If elimination of the deficient X chromosome is due to the deficiency itself then the percentage of eliminations should be equal in both classes.* Accordingly, Blond males were mated to females with morphologically normal chromosome constitution homozygous for singed-3. Elimination of the deficient chromosome was deduced from the appearance of not-Blond, singed setae mainly on head or thorax. Out of 323 Blond-Minute females (2) 143 had one or more such mosaic spots making a total of 167 (to which should be added 12 spots of somewhat different constitution cf. table 9). In 923 Blond not-Minute sisters (1) not a single spot was exhibited. This result proves that the deficient consitution of the Blond X chromosome itself is not the cause of eliminations, and suggests that eliminations of the X chromosome are due to the phenotypic "Minute reaction" (SCHULTZ 1929), that is, to the same or part of the same physiological condition which results in the development of a short-bristled late hatching "Minute" fly.

One difficulty, however, remained. Why should the "physiological Minute condition" eliminate only the deficient X chromosome? That only the deficient X was affected and not its normal partner seemed to be indicated by an experiment in which the sn^3 gene was located together with Bld in the deficient X, the other X containing white (*w*, I, 1, 5) or other recessives. In 221 def. Bld sn^3/w; normal II/normal II Minute females, 94 bristles containing spots on head and thorax were found, all not-Bld, not-sn^3. They seemed to result from eliminations of the deficient chromosome, while the absence of sn^3 spots seemed to exclude the elimination of the not-deficient X chromosome. Was there such an interaction that the physiological Minute condition produced by an uncovered deficiency in the X would eliminate just this deficient chromosome which was quite

stable when its phenotypic effect was suppressed by the normal allele for that region translocated to the second chromosome?

Autosomal Minutes and sex-linked spots

When the ability of autosomal Minutes to produce "autosomal" mosaics had been discovered, the following cross was made in order to detect a possible influence of such Minutes on the appearance of sex-linked mosaics: sn^3/sn^3 ♀ by $+^{sn}$; $My/+$ ♂ (*My*, Minute-y, III, 40.4). While 1020 $+^M F_1$ females had 3 single-bristle singed spots, 811 *My* F_1 sisters exhibited 16 spots (13 single-bristle, 3 larger ones, table 2a, first row). One of the spots in a $+^M$ fly occurred on the abdomen and is not included in the table. This result showed (1) that mosaic spots appear as rare occurrences even in not-Minute flies (BRIDGES in MORGAN, STURTEVANT, BRIDGES 1929, has encountered four such cases in his experiments) and (2) that an autosomal Minute increases the frequency of these occurrences, that is, is able to influence the fate of an X chromosome.

Later work confirmed this finding and has led to the use of autosomal Minutes as tools in the study of sex-linked mosaics.

Besides *My* three other autosomal Minutes have been tested for their effect on the X chromosome behavior, namely *Mw* (Minute-w, III, 80±), *M33j* (Minute-33j, III, 40.4) and *Mβ* (Minute-β, III, 85.4). Table 2a shows the results of some tests. It should be pointed out that the constitution of the X chromosomes varied in these experiments, so that the frequencies of spots in different experiments are not comparable. In some experiments only the head and thorax were inspected for spots. In all later tests the abdomen was inspected also. Due to the comparatively small size of the head and the low number of setae the number of head spots has been added to the number of thorax spots under one grouping. An inspection of tables 2a, b shows, among other results:

(1) The frequency of sex-linked spots in not-Minute flies varied from 0.0 to 6.0 on the head-thorax region and from 4.6 to 20.0 per cent on the abdomen. The frequency in Minute flies varied from 0.0 to 22.3 in the head-thorax region and from 8.0 to 36.6 per cent on the abdomen.

(2) A positive correlation is indicated between percentage frequency of head-thorax and abdominal spots:

(a) In not-Minute flies

experiment	*Mw*(5)	*Mβ*	*M33j*(3)	*M33j*(2)	*Mw*(4)	*M33j*(1)
frequency on head-thorax	0.0	0.7	0.8	1.4	4.5	6.0
frequency on abdomen	15.0	4.6	8.2	9.2	11.7	20.0

(b) In Minute flies

experiment	$Mw(5)$	$M\beta$	$Mw(4)$	$M33j(3)$	$M33j(1)$	$M33j(2)$
frequency on head-thorax	0.0	3.1	4.4	5.7	16.8	22.3
frequency on abdomen	27.1	8.0	28.1	33.4	36.6	15.9

Two of the discrepancies in these series seem to be based on a special condition which hindered the appearance of all head-thorax spots. This experiment ($Mw(5)$) therefore is not comparable with the rest.

(3) The relative increase of spots in Minute as compared to not-Minute flies varies from 1.0 to 15.6 times in the head-thorax and from 1.7 to 4.1 times in the abdominal region.

(4) The average increase is distinctly lower in the abdominal region.

(5) As far as the data are significant, no correlation seems to exist between amount of increase of spots in the two different body regions:

experiment	$Mw(4)$	$M33j(1)$	$M\beta$	$M33j(3)$	$M33j(2)$
increase on head-thorax	1.0	2.8	4.7	7.2	15.6
increase on abdomen	2.4	1.8	1.7	4.1	1.7

The data of tables 2a,b had shown the effect of autosomal Minutes on the *frequencies* of occurrence of sex-linked spots. Is there also an influence

TABLE 2a

Frequency of sex-linked spots in Minute and not-Minute females.

MINUTE USED AND NO. OF EXP.	INDIVIDUALS INSPECTED		HEAD-THORAX SPOTS					ABDOMINAL SPOTS				
			NO.		%		%	NO.		%		%
	+	M	+	M	+	M	M:+	+	M	+	M	M:+
My†	1020	811	2	16	.2	2.0	10.5	—	—	—	—	—
Mw (1)†	964	813	8	23	.8	2.8	3.4	—	—	—	—	—
(2)†	377	284	7	17	1.9	6.0	3.2	—	—	—	—	—
(3)†	151	119	3	20	2.0	16.8	8.5	—	—	—	—	—
(4)	154	114	7	5	4.5	4.4	1.0	18	32	11.7	28.1	2.4
(5)‡	432	247	0	0	0	0	—	65	67	15.0	27.1	1.8
M33j (1)	135	131	8	22	6.0	16.8	2.8	27	48	20.0	36.6	1.8
(2)	349	251	5	56	1.4	22.3	15.6	32	40	9.2	15.9	1.7
(3)*	377	296	3	17	.8	5.7	7.2	31	99	8.2	33.4	4.1
Mβ	307	227	2	7	.7	3.1	4.7	14	18	4.6	8.0	1.7

† Abdomen not inspected.

‡ No head-thorax spots present.

* No head spots present.

on *time* of occurrence in development of the process which leads to appearance of a mosaic spot? The earlier this time is, the larger the spot should be. Accordingly, in table 3 the spots are divided into three groups, those

TABLE 2b

Proportion of number of head-thorax:abdominal spots in $+^M$ and M females.

EXP.	NUMBER OF HEAD-THORAX AND ABDOMINAL SPOTS +	*M*
Mw	7– 18	5– 32
M33j (1)	8– 27	22– 48
(2)	5– 32	56– 40
(3)	3– 31	17– 99
Mβ	2– 14	7– 18
Total	**25–116**	**107–237**

χ^2 about 10. P<.01.

comprising only one seta, only two setae, and more than two setae. This last class includes all spots from those involving three setae up to those occasional spots which include all setae of the imaginal disc concerned. The data are comparatively meager, but the following generalizations seem to be warranted:

(1) Most experiments agree in the relative frequencies of small and larger spots in that the one seta spots form the majority both on the head-thorax and on the abdominal region. (2) Some striking exceptions to this occur, notably the head-thorax spots in Minute flies of *Mw*(2) and (3) and the abdominal spots in both not-Minute and Minute flies of *M33j*(1). Here the frequencies of spots larger than one seta is higher than that of one seta spots: 14:3; 16:4; 20:7; 36:22. (3) The proportion of

TABLE 3

Frequency of sex-linked spots of different sizes in the experiments described in Table 2a.

EXP	HEAD-THORAX								ABDOMEN							
	NO. OF SPOTS		SIZE OF SPOTS						NO. OF SPOTS		SIZE OF SPOTS					
	+	*M*	+			*M*			+	*M*	+			*M*		
			1	2	>2	1	2	>2			1	2	>2	1	2	>2
My	2	16	2	—	—	13	1	2	—	—	—	—	—	—	—	—
Mw (1)	8	23	5	—	3	16	2	5	—	—	—	—	—	—	—	—
(2)	7	17	3	2	2	3	3	11	—	—	—	—	—	—	—	—
(3)	3	20	3	—	—	4	3	13	—	—	—	—	—	—	—	—
(4)	7	5	5	—	2	4	—	1	18	32	17	—	1	13	10	9
(5)	0	0	0	0	0	0	0	0	65	67	41	17	7	42	11	14
M33j (1)	8	22	6	1	1	13	4	5	27	48	7	6	14	22	11	25
(2)	5	56	5	—	—	42	4	10	32	40	17	4	11	25	6	9
(3)	3	17	3	—	—	16	—	1	31	99	19	6	6	60	17	22
Mβ	2	7	2	—	—	2	—	5	14	18	9	2	3	9	2	7

single seta spots is lower in the abdomen than in the head-thorax region. (4) In most experiments no striking influence of the Minute on the time of occurrence of the spot-producing process can be found.

The ontogenetic meaning of some of these findings will be dealt with later.

The specificity of the effects of sex-linked and autosomal Minutes

In view of the effect of autosomal Minutes on the X chromosome it seemed desirable to test for a possible influence of an X chromosome Minute on the behavior of autosomes. The Blond Minute is known to be one of the most potent factors for the production of sex-linked spots and accordingly was chosen for the test in regard to autosomal spots. Females of the constitution *h st cu sr* e^s *ca* (located over most of the length of chromosome III) were mated to Blond males and their Minute daughters were inspected for a condition mosaic for *h*, *st*, *sr*, e^s or *ca*. Although the 413 F_1 females exhibited on head and thorax 94 mosaic conditions for Blond, none was found to possess an autosomal spot. This shows that the influence of Blond-Minute on the production of autosomal mosaics must be very slight, if it exists at all.

This fact is significant. For if one makes a general "physiological Minute condition" responsible for the occurrences of spot-producing processes one might expect to find a corresponding seriation of different Minutes in respect to their potencies to produce both sex-linked and autosomal spots. However, a seriation in respect to frequency of sex-linked mosaics would show roughly:

$$\text{Blond-Minute} > Mn > M33j,\ Mw,\ My$$

while the same Minutes (*Mn* excluded) with respect to frequency of autosomal mosaics probably would have to be arranged as:

$$Mw,\ My > M33j,\ \text{Blond-Minute}$$

The two seriations are given after an analysis of the data presented in different tables of this paper. Too great reliance cannot be attributed to details of these arrangements, as the experiments were carried out over a period of years and under different genetic and environmental conditions. Therefore no special table has been made up from these data, as the quantitative results might indicate a higher degree of accuracy than they really represent. However, there seems no doubt as to the validity of the main result, namely that the effect of Minutes on the frequencies of sex-linked and of autosomal spots varies independently so that one Minute factor may affect strongly the number of sex-linked but only slightly the number of autosomal spots and vice versa.

An even more striking correlation between certain autosomal Minutes and areas mosaic for definite regions of the same autosome will be presented in the chapter on "Autosomal spots."

THE MECHANISM OF MOSAIC FORMATION

Various hypotheses

In the foregoing pages the effect of Minutes on the process of mosaic formation was discussed in general terms. The following part will contain an analysis of the process itself.

BRIDGES' work with females carrying *Mn* in one X and recessive genes in the other seemed to have established (1) that the cells of a spot do not

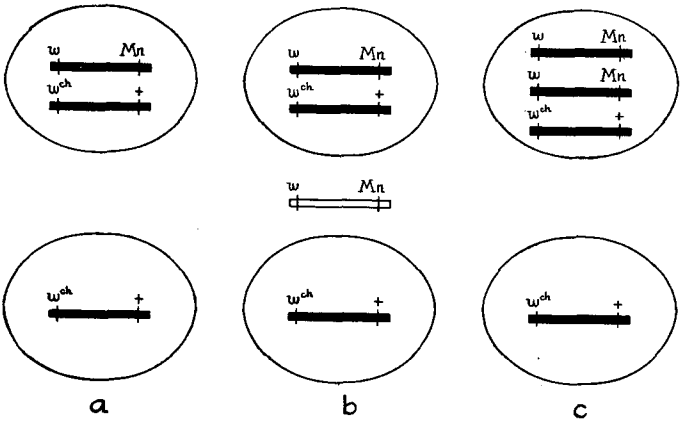


FIGURE 1 a–c. Three possibilities to account for elimination of an X chromosome.

contain the *Mn* chromosome and (2) that these cells are male in constitution, containing only one X with the recessive genes. Three main possibilities suggested themselves as mechanisms for the elimination of the *Mn* chromosome from the cells of the spot: (a) during a somatic division the *Mn* X chromosome does not divide and consequently passes into one daughter nucleus, leaving the other one in possession of only a division product of the not-*Mn* X chromosome; (b) the *Mn* X chromosome divides into two halves, but only one half passes into one of the daughter nuclei. The other half lags behind, is not included into a daughter nucleus, and degenerates in the cytoplasm; (c) the *Mn* X chromosome divides into two halves. These halves do not disjoin but pass together into one daughter nucleus.

The constitution of the daughter nuclei according to the three hypotheses is pictured in figure 1. No way of distinguishing between the mechanisms (a) and (b) was found, but a test between (a) and (b) on one side and (c) on the other seemed possible. The sister cell of the "elimination

cell" in (a) and (b) would be of the same constitution as the original constitution of the fly, while in (c) it would have a different constitution, being triplo-X. By using suitable gene markers such a condition as (c) could be demonstrated if it occurred. Accordingly females were bred which had white in their *Mn* chromosome and cherry (w^{ch}) in the other X chromosome. The eye color in such females is a light cherry. Their eyes were searched for spots of normal, dark cherry color, indicating the elimination of the *w Mn* chromosome. When such spots were found it was determined whether their surroundings in the eye were of the w/w^{ch} coloration as expected according to (a) and (b) or whether they contained a very light cherry spot, indicating the $w/w/w^{ch}$ constitution expected according to (c). On 1702 w *Mn*/w^{ch} females which were inspected 20 eye spots were found. They all were cherry colored and unaccompanied by a twin spot of $w/w/w^{ch}$ coloration or of the irregular facet arrangement characteristic for 3X+2A eyes. Sixteen of these spots covered an area of at least 3 or 4 but not more than about 50 facets; 4 spots included more than 150 and less than 300 facets. These facet numbers have not been determined accurately but have been estimated from sketches. The total number of facets in one eye is between 700 and 800 in females. Similarly, in 168 w^{ch}*Mn*/w females, two white eye spots were observed not accompanied by a twin spot of the darker colored triplo-X constitution w^{ch}*Mn*/w^{ch}*Mn*/w. These results rule against the hypothesis (c) provided that cells with two *Mn* factors and one normal allele are viable and can give rise to cell patches large enough to be recognized as mosaic spots. Nothing was known originally about this point.

Somatic segregation

y/sn^3 flies; preliminary discussion

The solution was brought about by chance. When it had been found that the "Minute condition" caused by an "uncovered" Blond-deficiency was necessary for the elimination of the deficiency X chromosome, an experiment was made in order to determine whether a deficiency which in itself does not produce a Minute effect would, together with an independent Minute factor, bring about the elimination of the deficient chromosome. The deficiency chosen was the well known Notch-8 (N^8) in the X chromosome and the Minute was the autosomal *Mw*. The following cross was made: N^8/y *Hw* dl-49 ♀ by sn^3; *Mw*/+ ♂, and the frequencies of head-thorax spots in N^8 and not-N^8 sister females were compared (table 4). The presence of *Hw* and of the dl-49 inversion is irrelevant. From 280 $N^8/sn^3;+^M$ or *Mw* females 9 mosaic spots were obtained while 381 control y *Hw*/dl-49/$sn^3;+^M$ or *Mw* females yielded 15 spots. Thus the frequency

of spots in N^8 was 3.6 per cent, in controls 3.9 per cent. There was no interaction of the Notch deficiency with the autosomal Minute-w.

The nine spots in the N^8/sn^3 flies were recognized by singed setae occurring presumably as a consequence of elimination of the N^8 chromosome. In cases where the sn^3 containing X chromosome would have been eliminated, no visible spot would have been produced, for the N^8 chromosome contained no recessive genetic marker which would have expressed itself in a spot (provided that a cell containing only the Notch-deficient X chromosome is able to reproduce sufficiently to give rise to a large enough cell-patch).

TABLE 4

N^8/y Hw dl-49 by sn^3; Mw/+. Head and thorax spots only.

	N^8		$+^N$	
	$+^M$	*Mw*	$+^M$	*Mw*
y spots	—	—	1	1
sn^3 spots	4	5	0	2
$\left.\begin{matrix} y- \\ sn^3 \end{matrix}\right\}$ twin spots	—	—	2	9
Total spots	**4**	**5**	**3**	**12**

The situation was different in the *y Hw* dl-49/sn^3 control flies. If there was no preference which of the two X chromosomes would be eliminated, then two different types of spots might be expected to occur in about equal numbers: (1) *y* spots in case of elimination of the sn^3 X chromosome and (2) sn^3 spots in case of elimination of the *y Hw* dl-49 X chromosome. The 15 spots found consisted of (1) two *y* spots and (2) two sn^3 spots while (3) the remaining 11 spots showed an unexpected structure: *they were twin-spots formed by a yellow not-singed area adjacent to a singed not-yellow area.*

The obvious explanation is that, during a somatic division of one of the cells of these 11 *y Hw* dl-49/sn^3 females, a segregation had taken place whereby one daughter cell obtained the yellow gene carried originally by one of the X chromosomes while the other cell obtained the singed gene carried originally by the other X. A process similar to gametic segregation of genes lying in opposite members of a pair of chromosomes had occurred in a somatic cell. The further division and normal somatic differentiation of the two daughter cells finally gave rise to mosaic twin areas.

These findings of somatic segregation suggested that the so-called chromosome elimination in certain cells leading to the appearance of mosaic spots was in all or most cases the consequence of somatic segrega-

tion. This theory is substantiated by three facts. (1) Further experiments demonstrated the general occurrence of twin spots in flies of suitable constitutions. (2) In appropriate experiments it could be shown that nearly all mosaic spots exhibit the results of somatic crossing over. This makes simple elimination hypotheses improbable. (3) The theory solves the difficulties encountered by the assumption that the sex-linked Minutes eliminate only their own chromosomes.

We shall first discuss point (3). The more important statements (1) and (2) will be dealt with in later sections.

Minute-n and Blond-Minute "elimination" as somatic segregation

Somatic segregation of the X chromosomes in a female carrying *Mn* in one X and a recessive gene in the other will lead to two daughter cells, one containing only *Mn*, the other only the recessive. *Mn* is known to be lethal to a male or a homozygous *Mn* female zygote. If we assume *Mn* in such a condition to be lethal to a somatic cell also we shall expect the one daughter cell to die while the other one, containing only the recessive, will give rise to the observed spot. (It might be argued that a cell containing only *Mn* is viable but phenotypically not different from the non-segregated surrounding tissue. This, however, is excluded by having a recessive gene together with *Mn* in the one X chromosome but not in the other X. Segregation without lethal effect of the *Mn* segregation product should exhibit *Mn* spots which also show the recessive gene effect. In the experiments discussed on p. 635 the *Mn* chromosome contained a white or cherry gene, but no spots showing the respective eye colors were found. Other experiments of similar nature are described in later sections of this paper.)

Somatic segregation can account also for the single spots in "Bld-Minute" flies. In heterozygous Bld-Minute females it will lead to a "not Bld-Minute" and a "Bld-Minute" cell. The former will give rise to a spot with not "Bld-Minute" phenotype while the latter is expected to die, that is, if one assumes a cell not to be viable in case it contains a completely uncovered X chromosome deficiency. Male zygotes containing the uncovered Bld-deficiency X chromosome or female zygotes homozygous for such a condition are known to be not viable.

The reader might be inclined to strengthen these arguments by pointing to DEMEREC'S studies (1934) on cell lethals. This, however, would not be justified as DEMEREC'S interpretation is *based* on the acceptance of the theory of somatic segregation and therefore cannot be used to prove this theory.

The influence of a Minute condition on the occurrence of mosaic spots thus consists in an increase of the tendency to somatic segregation. The

question asked at the close of the chapter on mosaic spots in Bld-Minute flies can now be answered. "Is there an interaction of such a kind that 'the physiological Minute condition' produced by an uncovered deficiency in the X chromosome would eliminate just this deficient chromosome?" The answer is this: elimination is not restricted to one X chromosome, but somatic segregation of the two X chromosomes leads to the appearance of spots which possess the non-deficient chromosome only.

Further analysis of somatic segregation in y/sn^3 flies

In order to justify the assumption of somatic segregation as a general cause of the occurrence of mosaic spots we have here to deal with those cases of table 4 which did not show a twin condition although such a condition might have been expected, for whenever a cell contains in each of its two X chromosomes one or more recessive, heterozygous genes capable of producing their phenotypes in small surface spots, the simple process of segregation will yield two neighboring cells pure for the genes of their respective segregated chromosomes. Why then did only 11 out of 15 cases show the twin spot condition? The following considerations have to enter into an answer to this question. (a) Even under the assumption that somatic segregation always leads to two sister cells which are both pure for originally heterozygous recessive genes, the appearance of the mosaic region will depend on the time of the segregation process in ontogeny. With a very late occurrence in development only two small twin areas could be produced, each consisting of only one cell if segregation took place during the very last division of the cells of the imaginal disc. In such cases it will frequently happen that one of the segregation products will form a seta and thus be recognized by its singed shape or yellow color, while the other segregation product will not happen to build up such a part of the hypodermis as will give rise to a seta and thus will not be recognizable. In other words, small twin spots will be liable to be recognized only in one of the segregation products. In large spots the probability is correspondingly higher that the areas formed by both segregation products will contain setae. They should therefore show the twin condition. (b) Even in larger spots a twin condition will be found lacking in case the cell descendants of one of the segregation products happen to become located in a region bare of setae, as for instance most of the scutellum, large areas on the head, the sternopleurae, the regions laterally from the posterior dorsocentrals. Twinning will be absent also in cases where the survival or reproductive ability of one of the daughter cells of the segregating division has been impaired, be it by chance, by normal developmental determination, or by lower genetic viability. To sum up the preliminary discussion: Small spots are expected to be often "singles," not twins; large spots in the majority of cases should be twins.

Tables 5 and 6 contain data pertinent to this question. Four different but fundamentally similar groups of experiments are summarized. In experiment 1 the females were of the constitution *y Hw* dl-49/sn^3 with or without *Mw*. They include, together with others, the flies discussed at the beginning of the present chapter. It is seen (table 5) that 23 out of 38

TABLE 5

Kinds and sizes of spots in experiments involving primarily y and sn^3 when located in opposite chromosomes.

EXP.	CONSTITUTION	INDIVID.	SPOTS	*y* SPOTS			sn^3 SPOTS			*y*-sn^3 TWIN SPOTS	
				NO. OF SETAE			NO. OF SETAE			NO. OF SETAE	
				1	2	>2	1	2	>2	2	>2
(1)	*y Hw* dl-49/sn^3	551	38†	2	—	2	8	1	2	4	19
(2)	*y w* (or w^e)/sn^3	376	212‡	40	9	12	62	12	10	7	60
(3)	*y* g^2bb/sn^3bb^z (a)	635	6†	—	—	1	5	—	—	—	—
	(b)	635	157*	22	8	6	60	14	9	9	29
(4)	*y bi cv* ct^6 *v* g^2 bb^1/sn^3bb^z (a)	214	19†	1	—	—	5	4	8	—	1
	(b)	214	73*	16	4	1	12	7	15	3	15
				81	21	22	152	38	44	23	124
	Totals	**1776**	**505**		**124**			**234**			**147**

† Head and thorax spots only.
‡ Head, thorax and abdominal spots.
* Abdominal spots only.

spots were twins and that 10 out of the 15 single spots were so small as to include only one seta, which obviously makes it impossible for them to show a twin condition. Of the 28 spots covering two or more setae the great majority, namely 23, were twins. Experiment 1 then corresponds closely to our expectation. In experiment 2 the constitution of the flies was *y w*/sn^3 or *y* w^e/sn^3 (*w* and w^e will be disregarded here). In addition, all individuals contained *M33j*. There were 67 twin spots out of a total of 212, and 102 out of the 145 not-twin spots were single seta spots. Of the 110 spots covering two or more setae 67 exhibited the twin condition, and 43 did not. But 21 of these 43 were so small as to include only two setae, thus still making it probable that the supposed twin area did not happen to cover a setae-forming region. Although the results in experiment 2 did not come as near to expectation as in 1 the agreement can be regarded as sufficient. The results will be further discussed after a description of experiments 3 and 4. In these a high frequency of spotting was induced by making the flies homozygous for recessive, mutant alleles of bobbed. In 3 one allele was the standard *bb*, the other one either the same or a very similar one; in 4 one was the lethal allele bb^1 the other was as in 3. Bobbed

can be called a recessive Minute gene, so that the flies in these experiments were under the influence of a "physiological Minute condition," which caused the high spotting frequency. Again the presence of other genes besides *y* and *sn*^{3} will be disregarded at this point. In 3 only 38 out of 163 spots were twins, but a consideration of the different sizes of spots again shows that 87 out of the 125 single spots were so small as to include only one seta. Of the remaining 38 single spots, 22 were so small as to include only two setae, but of the 76 spots covering two or more setae, there were 38 twin spots. Experiment 3 then, although showing a general agreement with expectation seems to deviate more from it than the two experiments 1 and 2. Before discussing this we shall consider experiment 4. Here 19 out of 92 spots were twins, and 34 out of the 73 single spots included only one seta. Of the remaining 39 single spots, 15 were so small as to include only two setae. Out of the total of 58 spots covering two or more setae, 19 exhibited the twin condition. As in experiment 3 these results seem to be in general, but not very close, agreement with the theory of simple somatic segregation as the cause of spotting.

TABLE 6

Further data on the size of spots in experiments 1–4 of table 5.

	(1)	(2)	(3)	(4)
Total *y* setae in single spots	137	182	69	26
Total *sn*^{3} setae in single spots	113	145	121	183
Total *y* setae in twins	121	230	64	27
Total *sn*^{3} setae in twins	80	208	86	45
Average *y* setae in twins	5.3	3.4	1.7	1.4
Average *sn*^{3} setae in twins	3.5	3.1	2.3	2.4
Average (*y*+*sn*^{3}) setae in twins	8.8	6.5	4.0	3.8

Table 6 summarizes certain facts which help to explain the apparent discrepancies. The last horizontal line shows that the average size of the twin spots in experiment 1 was more than double that of the twin spots in 3 and 4 while it was intermediate in 2, and that in 3 and 4 the average size of one of the twin areas was only of the order of magnitude of two setae. It follows that there was a considerably higher chance for the small twin areas in 3 and 4 to appear phenotypically only as single spots than there was for the large areas in 1 and 2. Part of the deviations from our expectation are further cleared up by the following considerations. If single spots are really parts of twin areas in which only one area had the opportunity to exhibit its phenotype, then if chance alone determined which of the two areas covered a seta-forming region one should expect an equal frequency of *sn*^{3} and of *y* single spots. An inspection of table 5, however, shows that,

at least in 3 and 4, there is no 1:1 ratio of the two types of spots. In different form this can be seen from the first two lines of table 6 where the total

TABLE 7

List of all 124 twin spots >2 from table 5 with number of y and sn^3 setae affected. Each horizontal line is the record of one kind of spot. The numbers in parentheses indicate the frequency with which this type of spot was represented. No number in parentheses was given when there was only one spot of its kind.

EXP. (CF. TABLE 5)	NO. OF SETAE y	NO. OF SETAE sn^3	DIFFERENCE: NO. y–NO. sn^3
(1)	3	1	+ 2
	28	1	+27
	(2) 1	2	− 1
	(3) 3	2	+ 1
	4	2	+ 2
	(2) 1	3	− 2
	3	3	0
	4	3	+ 1
	24	3	+21
	5	4	+ 1
	3	5	− 2
	5	5	0
	5	9	− 4
	1	14	−13
(2)	(7) 2	1	+ 1
	(2) 3	1	+ 2
	(2) 4	1	+ 3
	5	1	+ 4
	10	1	+ 9
	50	1	+49
	(4) 1	2	− 1
	(4) 2	2	0
	(3) 3	2	+ 1
	5	2	+ 3
	(5) 1	3	− 2
	(3) 2	3	− 1
	(2) 3	3	0
	4	3	+ 1
	(2) 5	3	+ 2
	(2) 6	3	+ 3
	(4) 1	4	− 3
	2	4	− 2
	(3) 3	4	− 1
(2) (cont'd)	(2) 1	5	− 4
	2	5	− 3
	4	5	− 1
	6	6	0
	1	7	− 6
	15	7	+ 8
	3	7	− 4
	1	10	− 9
	11	14	− 3
	1	15	−14
(3)	(5) 2	1	+ 1
	5	1	+ 4
	(6) 1	2	− 1
	(2) 2	2	0
	(2) 3	2	+ 1
	1	3	− 2
	(2) 2	3	− 1
	1	4	− 3
	(2) 2	4	− 2
	3	4	− 1
	4	4	0
	(2) 1	5	− 4
	2	5	− 3
	3	7	− 4
(4)	(2) 2	1	+ 1
	3	1	+ 2
	(5) 1	2	− 1
	2	2	0
	(3) 1	3	− 2
	3	3	0
	1	4	− 3
	2	5	− 3
	1	6	− 5

number of y and sn^3 setae has been noted. It is evident that the y setae are in the minority in 3 and 4, while there is a deficiency of sn^3 setae in 1 and especially in 2. Possible causes for these new discrepancies can be learned

from the second part of table 6. Here the frequencies of sn^3 and y setae are listed for all twin spots, that is, for all cases in which there is no doubt as to the occurrence of somatic segregation. The relative frequencies of the two types of setae in twin spots vary in the same direction as the total frequencies in single spots. Obviously, in these experiments, the chances for survival and reproduction of the two daughter cells from a segregating division were not equal. The vitality of y cells was lower than that of sn^3 cells in 3 and 4 but higher in 1 and 2. The greater the inequality in survival value, the higher the proportion of spots even of larger sizes which should appear only as single spots, the twin area having died or been kept small. This is roughly borne out by the data. What the causes of lowered viability were in these experiments cannot be determined accurately now. The presence of different alleles of bobbed in the two X chromosomes of 4 and possibly 3 has nothing to do with it, as will be shown later. However, it is suggestive that the cells in 4 which are pure for y are also pure for *bi*, ct^6, *cv*, *v*, and g^2. As it is known that this multiple mutant condition considerably lowers the viability of a whole individual, the assumption seems justified that a similar effect may be found also in mosaic parts.

Table 7 has been added to give a more complete representation of all twin spots with more than 2 setae which occurred in these experiments. It is interesting that such great inequalities of the two twin areas were observed as the case in which y area covers 50; sn^3 area covers 1; or y area has 24 setae and sn^3 area 3; or y area 1 and sn^3 area 15 seta.

Some of these very unequal twin spots might possibly be regarded as two single spots of independent origin, lying next to each other, but this must be very exceptional. The frequency of spots in most experiments is low enough as to make rare the occurrence of more than one spot on an individual, although the incidence of flies with two or more spots is higher than according to chance (BRIDGES in MORGAN, STURTEVANT and BRIDGES 1929; also numerous data of the author). If several spots occur on one fly they have no tendency to be neighbors.

Summing up, it seems clear that somatic segregation does not only account for the occurrence of twin spots but also for that of single spots. But while the evidence in the cases of twin spots is direct, in the cases of single spots it is of such a nature as to leave open the possibility that not all single spots can be regarded as vestigial twin spots whose region did not happen to affect a seta. A numerical treatment of the data which theoretically should be able to give a final decision is not feasible on account of the many variable factors involved. The next section, however, will show that a certain proportion of single spots are to be expected which have never been partners of an original phenotypic twin group.

Somatic segregation and crossing over

Experiments involving *y sn*^{3}/+ flies

In the experiments summarized in table 5, *y* and *sn*^{3} were in opposite chromosomes. When both mutants were in the same chromosome, some new results were obtained (table 8): Three kinds of spots appeared with

TABLE 8

Spots in flies of the basic constitution y sn^3/+.

EXP.	CONST.	IND.	SPOTS	*y sn*^{3}			*y*			*sn*^{3}			OTHER SPOTS
				1	2	>2	1	2	>2	1	2	>2	
(1)	*y sn*^{3}*bb/car bb*	83	34	10	4	6	3	3	5	2	—	1	—
(2)	*y sn*^{3}*bb/car bb*	495	72	25	15	10	12	2	5	2	—	1	—
(3)	*y sn*^{3}*bb*/+†	508	35	10	11	4	5	4	—	1	—	—	—
(4)	*y sn*^{3}/+†	321	21	8	3	4	1	1	2	—	—	—	2*
				53	33	24	21	10	12	5	—	2	
Totals		**1407**	**162**		**110**			**43**			**7**		**2**

† Partly *Mw*/+.

* +^{y}+^{sn}; ♂-colored.

different frequencies, namely 110 *y sn*^{3}, 43 *y* and 7 *sn*^{3} spots. The finding of *y sn*^{3} spots was expected, for segregation in *y sn*^{3}/+ females (disregarding the presence of *bb* and *car*) should give rise to *y sn*^{3} and + cells, which would be visible as *y sn*^{3} spots. The occurrence of *y* and of *sn*^{3} spots needs an additional interpretation. Somatic crossing over between *y* and *sn*^{3} would separate these two genes from each other and thus afford an explanation. If the crossover process occurred during a two strand stage, the resulting strands would be *y*+^{sn} and +^{y}*sn*^{3}, and if segregation ensued a *y* and a *sn*^{3} twin spot would be produced. No twin spots were found, making the two strand crossing over assumption invalid. If, however, somatic crossing over occurred at a four strand stage between two of the four strands, and segregation two strands by two strands followed, then the facts can be explained (fig. 2). It is seen that following single crossing over, different types of chromatid segregation, namely **x** and **y**, give rise to either *y* or *sn*^{3} single spots. (Throughout this paper the term "chromatid" is used in reference to the strands which *constitute* a multivalent chromosome group during prophase and metaphase, as well as in reference to those chromosomes of anaphase and telophase which *originated* from a multivalent.)

If crossing over occurs at the four strand stage the subsequent segregation process will be expected to lead to either one of two results. (1) A separation of the four chromatids into two daughter cells will occur, followed

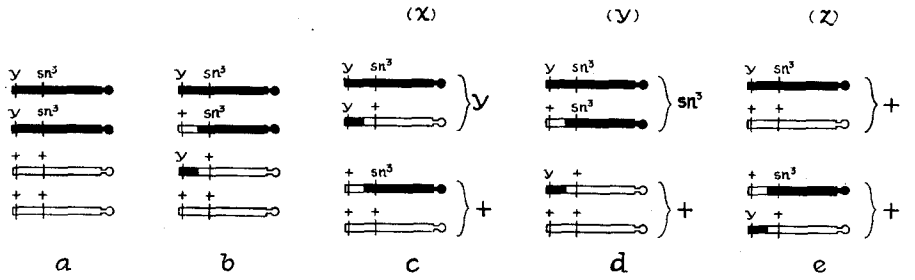


FIGURE 2. *y sn³/+*. Crossing over between *y* and *sn³* at a four strand stage. a. Non-crossover chromatids. b. Two crossover and two non-crossover chromatids. c-e. Three different types of chromatid segregation.

later by normal mitosis or (2) the initiated segregation will bring about a true reduction of chromatids leading to a second segregating division with resulting cells containing only single X chromatids. In females of the constitution *y sn³/++*, such a reduction process, after crossing over between two of four strands, should lead to four cells with the genes *y sn³*, *y+sn*, *+y sn³* and *++*. The areas resulting from later cell-divisions of the segregation products would exhibit the phenotypes *y sn³*, *y*, and *sn³*. The visible result would be a triple spot. Not a single spot of this nature was observed; the hypothesis of a complete somatic reduction process is thus refuted.

We can test the assumption of somatic crossing over at a four strand stage by applying it to the earlier experiments in which the constitution of the flies was *y/sn³*. Single crossing over between *y* and *sn³* at a two strand stage would yield *y sn³* and *++* strands; segregation would result in *y sn³* spots. No such spots have been found (table 5). Crossing over at a four strand stage, however, would, according to different types of chromatid segregation, produce *y* or *sn³* single spots (fig. 3). Such spots did occur and while many of them could be regarded as vestiges of potential twin spots, it is possible to assume a certain number of them to have been products of crossing over between *y* and *sn³* in the manner suggested.

Again it is obvious that no reduction of chromatids occurred which

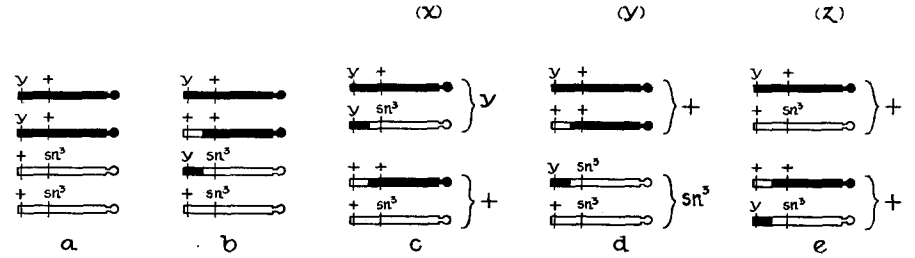
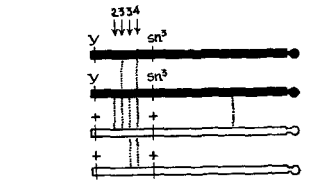
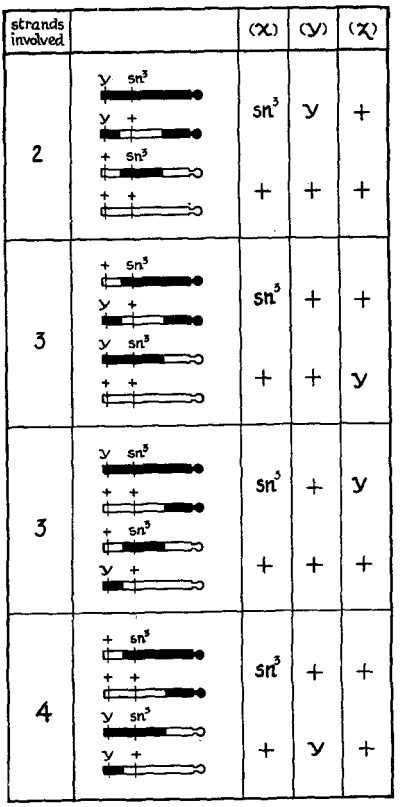


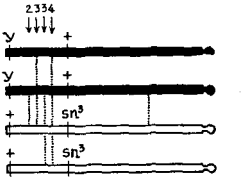
FIGURE 3. *y/sn³*. Crossing over between *y* and *sn³* at a four strand stage.

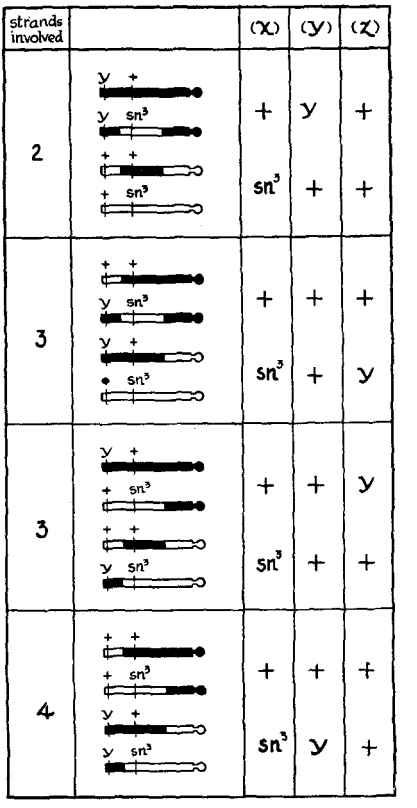
would have resulted in triple spots *y*, *sn*^{3}, and *y sn*^{3}. No triple spots were found and the phenotype *y sn*^{3} did not occur even in single spots.

If one characterizes the two types of chromatid segregation **x** and **y** by the constitution of the right chromatid ends, then **x**-segregation would be







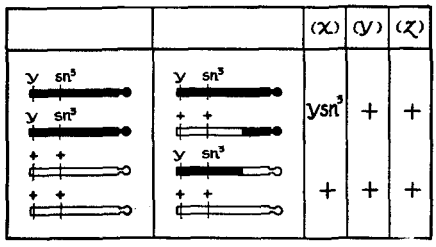


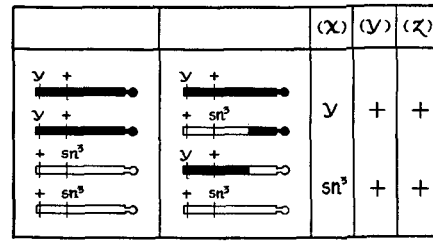
FIGURES 4 (LEFT) AND 5 (RIGHT). *y sn*^{3}/+ and *y*/*sn*^{3}. The four different types of double crossovers, involving 2, 3, and 4 chromatids and the results of the three different types of segregation.

equational and **y**-segregation reductional. As the right end of the X chromosome is known to contain the fibre attachment point, processes **x** and **z** would result from separation of sister attachment points while **y** would occur only when sister points stay together. It is possible to account for all observed spots with the assumption of equational segregation for the

right end, if one considers the possibility of occurrence of somatic double crossovers simultaneously to the left and to the right of *sn*^{3} (figs. 4, 5). Four different kinds of double crossover processes within the four strand "tetrad" are possible, involving two, three, and all four strands. After single crossing over **x**-segregation results in *y* single spots in flies of both constitutions *y sn*^{3}/++ and *y*+/+*sn*^{3}, after double crossing over it results in *sn*^{3} spots. If **y** segregation occurs, it gives rise to *y* spots in one-half of all cases; similarly **z** segregation produces visible *y* spots in two out of the four cases.

While a decision between the hypotheses of single crossing over and different kinds of segregation or single and double crossing over and equa-





FIGURES 6 (LEFT) AND 7 (RIGHT). *y sn*^{3}/+ and *y*/*sn*^{3}. Results of crossing over to the right of *sn*^{3}.

tional **x**- and **z**-segregation only cannot be derived from the present data, the second hypothesis seems to be in better agreement with the known facts in regard to the separation of daughter chromosomes in mitosis. We shall return to this question in the next chapter.

What is the frequency of the different crossover types in the *y sn*^{3}/+ experiments? We shall regard (and shall justify this hypothesis later) the *y sn*^{3} spots as due to single crossing over between *sn*^{3} and the right end of the X chromosome (fig. 6; see also fig. 7 for the *y*/*sn*^{3} flies). The *sn*^{3} spots are considered to be due to double crossing over. In determining the frequency of crossing over we cannot follow the usual procedure in germinal crossing over and base the calculation on the total of observed non-crossovers and crossovers, since the absence of crossing over in the great majority of somatic cells makes the number of non-crossovers rather meaningless. However, we can determine the relative frequencies of observed crossovers to the right and to the left of *sn*^{3} and the frequency of double crossovers. The frequencies of *y sn*^{3}, *y*, and *sn*^{3} spots were 110, 43, and 7. As was shown earlier, some *y* spots have to be regarded as products of **z**-segregation after double crossing over. As they constitute one-third of all visible double crossover products, their number is taken as 3.5. After subtracting this number from the total number of *y* spots, we find the proportion of spots produced after crossing over to the right of *sn*^{3} (*y sn*^{3} spots)

to spots from crossing over to the left of sn^3 (y spots) to be 110:39.5. Only the **x**-type of segregation in single crossing over yields visible spots, while the equally frequent **z**-type remains undetected. In double crossing over both **x**- and **z**-segregations result in visible spots, a total of 75 per cent of all cases. In order to have comparable figures we have to insert two-thirds of the number of visible double crossover spots, namely $(7+3.5).\frac{2}{3}=7$. Thus the proportion, single crossovers to the right of sn^3:singles to the left:doubles is 110:39.5:7. Comparing these values with the corresponding frequencies in meiosis, we find for the single crossovers 36:20.5. (MORGAN, BRIDGES, and STURTEVANT 1925, modified; no satisfactory data are available for the double crossovers.) In mitosis there seems to occur a higher proportion of all crossovers to the right of sn^3 than in meiosis. Even this frequency of somatic crossovers to the left of sn^3 was unusually high, as further experiments show.

Experiments involving Blond

In table 9 experiments are summarized in which somatic crossing over in females heterozygous for Blond, and either sn^3 or a forked (f, 56.7)

TABLE 9

Spots in Blond-Minute flies heterozygous for sn^3, f, or f^5; mainly head-thorax spots except in exp. (2).

EXP.	CONST.	SPOTS	TYPE OF SPOT*		
			$+^{Bld}+^{m}+^{M}$	$+^{Bld}{}_{m}+^{M}$	$+++-+f^5+$
(1)	$\frac{Bld\ +}{+\ sn^3}$	179	9(+3†)	167	—
(2)	$\frac{Bld\ +}{+\ f^s}$	178	8	168	2
(3)	$\frac{Bld\ sn^3}{+\ +}$	91	91	—	—
(4)	$\frac{Bld\ f}{+\ +}$	15	15	—	—

* m stands for either sn^3 or f; $+^M$ for "not Bld-Minute," i.e., long setae; "–" signifies a twin spot condition (+++ next to $+f^5+$).

† The three spots had $+^{Bld}+^{sn}$ setae of still smaller length than Bld-Minute.

allele was studied. The flies had two normal chromosomes II and were therefore of Minute type on account of the Blond deficiency in the X chromosome. In experiment (1) 167 out of 179 spots can be explained as

products of crossing over to the right of *sn*^{3}, for if such crossing over occurs, normal equational disjunction of daughter fibre points will produce two sister cells of the constitution Bld+/Bld+ and +*sn*^{3}/+*sn*^{3}. The former is assumed not to be able to give rise to a viable patch of cells, the latter multiplies to produce a +^{Bld} *sn*^{3} spot with normal sized setae. As can be readily seen from constructing a diagram for this case similar to our figure 7 for a previously discussed experiment, the other two mathematically possible types of disjunctions **y** and **z,** do not give rise to visibly new constitutions. However, crossing over to the left of *sn*^{3} and subsequent disjunction of sister attachment points (fig. 3) can lead to sister cells Bld+/Bld *sn*^{3} and ++/+ *sn*^{3} the latter of which result in normal sized, not-Bld, not-*sn*^{3} bristles. Nine such spots were found. Non-disjunctional segregation **y** of the fibre points would produce Bld *sn*^{3}/+*sn*^{3} bristles. No such case was found. Double crossovers which after normal disjunction of the fibre points (similar to figure 5) also would yield Bld *sn*^{3}/+*sn*^{3} spots besides half as many wild type spots were also absent. (There were three spots with not-Bld, not-*sn*^{3}, but shorter than Bld-Minute bristles. Their nature is not clear.)

In summing up experiment 1, we find the great majority of spots 167 out of 179, to be due to crossing over to the right and only 9 due to crossing over to the left of *sn*^{3}. This is a much lower proportion of left crossovers than in germinal crossing over and also considerably lower than in the experiment with *y sn*^{3}. That no case of double crossing over was encountered agrees with the rarity of left crossovers. A nearly identical result was obtained in experiment 2 which was of a similar nature as 1 except for the use of *f*^{5} instead of *sn*^{3}. In spite of *f*^{5} being 35.7 map units farther away from the left end than *sn*^{3} the proportion of left to right crossovers was still 8:168, disregarding the two twin spots in the last column. Again no double crossover types occurred. (One of the two twin spots covered the whole left mesothorax except the scutellum with a division line posteriorly from the anterior notopleural, presutural and anterior dorsocentral bristles which separated the anterior +^{f} part from the posterior *f*^{5} part; the other not quite decisive spot was formed by a left +^{f} ant. postalar and *f*^{5} ant. scutellar bristle. These spots can formally be explained by two crossover processes, first one to the left of *f*^{5}, resulting in a +/*f*^{5} cell, and a second one occurring in this cell or one of its near descendants and segregating +/+ and *f*^{5}/*f*^{5} twins.)

Summing up experiments 1 and 2, it is seen that the relative frequency of left crossovers is very low. Especially interesting in view of the occurrence of 17 cases of left crossovers with normal disjunction of the fibre points is the comp ete absence of left crossover cases with non-disjunction of the fibre points. As pointed out before, they would have been visible

TABLE 10*

Spots in flies containing y sn^3 and Mn.

In exp. (2) some of the individuals were of the constitution XXY (see p. 709).

In exp. (3) the not-Mn X chromosome carried besides y, the genes g^2 and ty (tiny bristles). The presence of the latter generally made a separation of Mn and $+^{Mn}$ spots impossible.

EXP.	CONST.	IND.	SPOTS	% SPOTS	+			$y\ sn^3$			y			sn^3			y-sn^3		+ ♂ COLOR
					1	2	>2	1	2	>2	1	2	>2	1	2	>2	2	>2	
(1)	$\frac{+\ sn^3\ Mn}{+\ +\ +}$	1038	29	2.8	5	2	8	3	—	—	3	2	1	3	—	—	—	—	2
	Totals						**15**			**3**			**6**			**3**			**2**
(2)	$\frac{y\ +\ Mn}{+\ sn^3\ +}$	349	254	72.8	3	—	1	—	—	—	33	6	1	60	23	112	3	4	8
	Totals						**4**						**40**			**195**		**7**	**8**
(3)	$\frac{+\ sn^3\ Mn}{y\ +\ +}$	1040	121	11.6	—	—	—	—	—	—	25	9	69	11	1	3	1	2	—
	Totals												**103**			**15**		**3**	
(4)	$\frac{+\ +\ Mn}{y\ sn^3\ +}$	827	96	11.6	—	—	—	25	13	51	4	1	—	2	—	—	—	—	—
	Totals									**90**			**5**			**2**			

* In exp. (1), the 3 y sn^3 spots and 2 of the sn^3 spots were probably *Mn*.
In exp. (2), 4 y spots were probably *Mn*, 103 sn^3 spots were $+^M$, and one sn^3 spot had very small setae.
In exp. (3), 10 sn^3 spots were probably *Mn*.
In exp. (4), 33 y sn^3 spots were $+^M$, one was probably *Mn*. The total of 90 y sn^3 spots includes one of unrecorded area. Of the y and the sn^3 spots one had small setae and one setae similar to $+^M$.

spots with Bld sn^3 (or Bld f^5) setae. Their absence shows that non-disjunction of the daughter fibre at least in these cases of left crossing over either does not occur at all or is rare.

In experiments 3 and 4 only one type of spot was found. This type corresponds in origin to most of the spots in 1 and 2 namely either to the single crossovers to the right or to the left crossovers, both with normal disjunction of the daughter fibre points. Each of these types gives rise to $++$ spots. Bld sn^3 (and $++$) spots would have been produced if single crossing over and non-disjunction of daughter fibres or if double crossovers had occurred. The absence of such spots agrees with the absence of corresponding spots in 1 and 2.

Finally, it is easily seen that no chromatid reduction has taken place, as certain types of twin spots should have occurred in all four experiments with Blond. Their absence is proof of segregation, two strands by two strands, without reduction.

Experiments involving *y*, sn^3, and *Mn*

The finding of somatic crossing over along the length of the X chromosome seems to contradict BRIDGES' (1925) results with spots in Minute-n flies. He states that no spots occurred which showed recessive characters determined by genes located in the *Mn*-carrying chromosome nor that spots occurred which showed only some, but not all, characters determined by genes in the other X. In the language of the crossing over theory this would mean no somatic crossing over occurred along the main length of the chromosomes, from *y* (0.0) to *Mn* (62.7). In order to clear up the apparent discrepancy, flies heterozygous for *y*, sn^3, and *Mn* were produced and scrutinized for spots. All four possible different distributions of these three factors and their alleles over the two X chromosomes were investigated. The results are assembled in table 10. In most spots no distinction could be made between *M* and $+^M$ seta length. Wherever this character could be classified it is recorded in the footnote. It is apparent that in each of the four experiments more than a single type of spot was found. However, in each case one type predominates. In the only case in which this prevalence amounts to less than 75 percent of all spots an external cause can be seen to be responsible, namely the most frequent class of spots in experiment 1 is distinguished from the surrounding tissue by its $+^M$ condition only. Spots of this constitution are recognizable, in general, only if they include one or more macrochaetae on the head or thorax, while all other spots exhibiting *y* or sn^3 or both *y* and sn^3 are recognizable anywhere and both in macro- and microchaetae. If only those spots are tabulated which have about equal chances of being recognized, namely head-thorax spots which include macrochaetae, their numbers are found to be

TABLE 11

Results of **x** *(equational) and* **y** *(reductional) segregation in experiments involving y, sn³, and Mn. In each case the upper symbols represent one segregate and the lower symbols represent its twin segregate.*

EXP.	SINGLE CROSSOVER IN REGION	SEGREGATION* (x)	(y)
(1)	1	*y Mn* *Mn*	dies +
$\frac{y\ sn^3\ Mn}{+\ +\ +}$	2	*y sn³ Mn* *Mn*	dies +
	3	dies +	*Mn* *Mn*
(2)	1	*y Mn* *Mn*	dies *sn³*
$\frac{y\ +\ Mn}{+\ sn^3\ +}$	2	*y Mn* *sn³ Mn*	dies +
		dies *sn³*	*Mn* *Mn*
(3)	1	*Mn* *y Mn*	dies +
$\frac{+\ sn^3\ Mn}{y\ +\ +}$	2	*sn³ Mn* *y Mn*	dies +
	3	dies *y*	*Mn* *Mn*
(4)	1	*Mn* *y Mn*	dies *sn³*
$\frac{+\ +\ Mn}{y\ sn^3\ +}$	2	*Mn* *y sn³ Mn*	dies +
	3	dies *y sn³*	*Mn* *Mn*

* z-segregation yields always: $\frac{Mn}{Mn}$

14+ and $2sn^3$, thus making experiment 1 fit in with the others as to the striking prevalence of one type of spot. In order to find out the processes by which the different types of spots were produced, table 11 was constructed; it gives the types of spots to be expected in case of single somatic crossing over in the regions (1) *y*-sn^3, (2) sn^3-*Mn* and (3) *Mn*-spindle fibre point and with **(x)** equational or **(y)** reductional segregation of the, daughter fibres. Another possible equational separation, **(z),** will not lead to visibly different spots. It is assumed that no chromatid reduction takes place. The contrary assumption can be disproved as in previous discussions.

An analysis has to be based on a consideration of *all* four experiments. Regarding the + spots in experiment 1 it is seen that three different processes of crossing over and segregation, namely **(1y), (2y),** and **(3x)** lead to + spots. As we expect the same processes in comparable frequencies to occur in experiments 2 to 4, we have to find out from table 11 what kinds of spots would result from these processes.

(a) Process **1y** would lead to sn^3 in experiment 2, + in experiment 3, and sn^3 in experiment 4. Indeed, experiment 2 yielded sn^3 spots as the largest class, in conformity with the + spots constituting the largest class in experiment 1. In experiment 3, however, no + spots were found and in experiment 4 the sn^3 spots constituted the smallest class. Thus process **1y** does not fulfill the requirements.

(b) Process **2y** can be excluded also. It would lead to + spots in all four experiments, while + spots actually form the smallest class in experiment 2 and do not occur at all in experiments 3 and 4.

(c) The remaining process **(3x)** would give results in accordance with all actual findings, as the different kinds of spots to be expected from it represent indeed the most frequent class in each experiment.

A similar analysis, carried out in regard to the less frequent classes of spots leads to the following results: Process **1x,** crossing over between *y* and sn^3 and equational fibre point segregation can account for *y* spots in experiments 1 to 4; process **2x,** crossing over between sn^3 and *Mn* with equational fibre point segregation can account for *y* sn^3 spots in experiments 1 and 4 and for *y*-sn^3 twin spots in experiments 2 and 3. It is seen in some cases that certain classes of spots are made up from products of different processes, as in the case of *y* spots in experiment 3, where the majority is considered to have originated in consequence of **3x,** while some must be products of **1x.** Furthermore, whenever *y*-sn^3 twin spots are expected, as in experiments 2 and 3, some of these will cover only so small an area as to appear as single *y* or sn^3 spots and will therefore add to the heterogeneity of the *y* or sn^3 classes. In some of these cases the different origin should lead to spots which contain either Minute-n or its normal

allele. As indicated in table 10, both types of spots were frequently found in the different classes and were present in proportions which were roughly in conformity with expectations.

There are a few types of spots which cannot be accounted for on the basis of single crossovers namely in experiment 1, 3 sn^3 spots, 2+ ♂ colored spots; in experiment 2, 4 + spots, 8+ ♂ colored spots; in experiment 4, 2 sn^3 spots.

A discussion of the + ♂ colored spots will be postponed (cf. p. 667.) The remaining few spots can be accounted for by double (and triple) crossing over, as can be readily seen. The occurrence of such multiple cross-

TABLE 12

Number of spots with 1 or 2 and with more than 2 setae in experiments 1–4 from table 10.

EXP.	PREVALENT CLASS		ALL OTHERS	
	1–2 SETAE	>2 SETAE	1–2 SETAE	>2 SETAE
(1)	7	8	11	1
(2)	83	112	45	6
(3)	33	69	13	5
(4)	38	51	7	0
	161	240	76	12
Totals	**401**		**88**	
% of spots >2	60		14	

overs should lead to certain other spots also which would not be distinguishable from the spots produced by the single crossover processes. This adds slightly to the heterogeneity of the main classes.

The following summary of the results of these tests is based on the as yet unproved assumption that apparent non-crossovers are really crossovers between the rightmost locus investigated (*Mn*) and the fibre point. We then find: (1) the most frequent cause of spots is somatic crossing over in the region nearest the fibre point, region 3, and equational segregation of the fibre points; (2) somatic crossing over in other regions of the X chromosomes occurs also and if followed by equational divisions of the fibre points accounts for the less frequent types of spots.

The relative frequencies of the different types of crossovers will be considered after one more result has been pointed out.

If we tabulate (table 12) for experiments 1 to 4 the numbers of spots which cover only 1 or 2 setae and those which cover more than 2 setae, we see that 60 per cent of all spots in the prevalent class and only 14 per cent of all other classes belong to the larger kind. The difference becomes more significant still if we remember that part of the spots in the main class belong in reality to the other group and thus should tend to increase the

number of small spots in the prevalent class. This striking correlation between chromosome region of somatic crossing over and size of spot might be accounted for on two different assumptions (other possible but less probable causes will not be discussed): (a) The products of crossing over to the left of *Mn* might have lower viability than those of crossing over to the right so that their growth is more restricted; (b) the relative frequency of crossing over to the left of *Mn* increases with the age of the developing insect, so that late cell divisions are more frequently preceeded by such kind of crossing over. As the size of the spot area formed decreases the later the time at which segregation takes place, crossovers to the left of *Mn* would result in a relatively high number of small spots.

Assumption (a) can be rejected, as there is no obvious reason why viability differences should differentiate between crossovers to the left or to the right of *Mn*, especially as certain spot constitutions which represent left crossovers in one experiment signify right crossovers in another. This leaves assumption (b) as an explanation of the average size differences of the different types of spots. *In Mn flies the region of somatic crossing over along the X-chromosome is correlated with the developmental stage of the organism.* No such relation was found in the not-*Mn* flies recorded in table 8.

A calculation of the relative frequencies of crossing over in different regions is made very difficult by this finding. It is probable that many cases of segregation after crossing over to the left of *Mn* occur so late that no seta is involved and the mosaic area remains undetected. The relative numbers of observed spots belonging (primarily) to the right and to the left crossover group is therefore biased to the disadvantage of the smaller left crossover spots. How great this bias is can hardly be estimated at present, but it seems very doubtful that it will be great enough to establish a ratio between somatic crossovers to the left and to the right of *Mn* which equals the ratio in germinal crossing over. If equality existed, the chance of observing a left crossover spot would have to be only 1/95 of that of observing a right crossover one, as the germinal ratio is of the order of 19:1 and the observed somatic ratio is of the order of 1:5 (88:401). If this chance is considered too small, it follows that somatic crossing over should be more frequent in the immediate neighborhood of the spindle fibre than in other regions of the X chromosomes. This finding agrees with the similar though less pronounced concentration of crossovers to the right of sn^3 observed in the experiments described in table 9 and table 8. However, such a calculation based on the *total* of observed spots obscures the fact that at different stages of development the relative frequencies of somatic crossovers in different chromosome regions vary.

The correlation between region of crossing over and developmental stage throws light on the divergence of our findings of more than one type of spot and the earlier findings of BRIDGES in which only one kind of spot, explicable by crossing over to the right of *Mn*, had been observed. BRIDGES focussed his attention on the larger spots, not on single seta spots; and he restricted his observation mainly to the head and thorax. As the great majority of spots originating after crossing over to the left of *Mn* comprises only 1 or 2 setae, it is probable that they have been overlooked formerly. Only 12 out of 489 spots summarized in table 12 covered more than 2 setae and only 3 of these occurred on head or thorax. It is not surprising that such a small number of aberrant spots should have gone unobserved.

There is one more point of divergence between the earlier results and interpretations of spots in *Mn* flies and the present ones. It relates to the number of X chromosomes present in cells of the mosaic area. This question will be considered separately.

Experiments involving *y*, *sn*^{3} and "Theta"

No proof has been given as yet for the statement that the apparent noncrossover spots were indeed results of crossing over to the right of the rightmost locus considered. This will be demonstrated now and it will be shown that somatic crossing over is a general process preceeding somatic segregation.

As indicated in the introduction, an experiment was undertaken to determine whether the whole chromosome was lost during the supposed elimination of the *Mn* chromosome. All former findings except those described in the last section had pointed to an "elimination" of at least most of the *Mn* X chromosome—from yellow (0.0) to the right of *Mn* (56.7). There were no "good" loci known to the right of *Mn* which could be tested with respect to their possible elimination. However, MULLER'S finding of an induced, cytologically visible duplication, Theta (θ), had provided a tool for this investigation (PATTERSON 1930). Theta is a deleted X chromosome comprising the left end with the loci yellow, scute, and broad (0.0–0.6) and bobbed from the right end. In the stocks used Theta was attached to the right end of a normal X. The chief property by which the presence of Theta is ascertained is its possession of a normal allele of yellow. In males or females in which the normal X chromosomes contain the recessive mutant yellow, the presence of Theta can be seen by their not-yellow normal body color.

When females of the constitution *y Mn* θ/*y* were inspected for spots of not-Minute setae, the color of these spots was expected to be yellow in case a complete elimination of the *y Mn* θ chromosome had taken place

and to be normal when only the *y Mn* part, but not the *θ* part of the chromosome had been lost. In a first experiment of this type 26 spots were found all not-yellow. This seemed to prove that only a part of the X was eliminated and that a break had occurred between *Mn* and the spindle fibre insertion, where Theta is attached. When, however, females of a similar constitution were examined in which the Theta-fragment was attached to the not-*Mn* chromosome (*y Mn*/*y θ*), it was discovered that the not-*Mn* spots were of two kinds, some being not-yellow, others being yellow. The first kind would be expected from a simple elimination of most or all of the *y Mn* chromosome. But the yellow spots seemed to show that *together with the Mn-containing part of one X chromosome the Theta part of the other X chromosome had disappeared.* A full analysis of these findings will be given in a later chapter. Here we shall follow the implications in regard to mosaics in which no sex-linked Minute is involved.

When somatic segregation had been recognized as the process leading to mosaic formation it was obvious that an interpretation of the Minute-Theta results involved assumptions of somatic crossing over to the right of *Mn*. Accordingly Theta was introduced into females of constitution otherwise similar to that described in the preceding chapter (the presence of *Hw* and dl-49 may be disregarded, but see p. 708). Table 13 contains the results. Two main types of females were inspected: (1) containing *y* and *sn*^{3} in one X and *y* and Theta in the other and (2) containing *y*, *sn*^{3} and Theta together in one X and only *y* in the other one. In these experiments due to the homozygous condition for *y* at the left end of the X chromosomes, crossing over between the X chromosomes, if it occurred at all, could be observed in its effect only when the exchange had taken place between the locus of *sn*^{3} and the attachment point of Theta. Three main possibilities seemed to exist: (a) no somatic crossing over, (b) somatic crossing over occurring at a two strand stage with respect to the two X chromosomes, each chromosome being still represented as a single strand, (c) somatic crossing over at a four strand stage. These might be regarded to be of unequal probability if considered in the light of our foregoing analysis and of cytological results. But the analysis offered a new test of the findings reported above and it was also thought best to judge the genetic evidence of these experiments on its own merit as much as possible. This will be done herewith.

(a) No somatic crossing over. In this case somatic segregation in experiment 1 should have yielded two daughter cells with the constitutions *y sn*^{3} and *y θ* giving rise to *y sn*^{3} spots (*y θ* spots are phenotypically indistinguishable from the surrounding tissue). Only two *y sn*^{3} spots out of 143 spots were found, showing that segregation without crossing over cannot account for the findings.

TABLE 13

Spots in experiments involving primarily y, sn³ and θ. Part of the individuals contained an autosomal Minute in order to increase the frequency of spots.

EXP.	CONSTITUTION	INDIVIDUALS	SPOTS	*sn³*			*y*			*y sn³*		*y-sn³*		*y sn³-sn³*	+♂ COL.
				1	2	>2	1	2	>2	1	>2	2	>2	>2	
1a	*y sn³/y θ*	170	53	16	5	20	3	2	—	1	1	—	2	1	2
b	*y sn³ bb/y Hw* dl49 *θ*	268	59	33	10	11	4	—	1	—	—	—	—	—	—
c	*y sn³ bb/y θ*	2558	31*	13	1	7	8	—	—	—	—	1	1	—	—
				62	16	38	15	2	1	1	1	1	3		
Totals Exp. 1		**2996**	**143**	**116**			**18**			**2**		**4**		**1**	**2**
2a	*y sn³ θ/y Hw* dl49	679+	148	87	28	21	6	2	3	—	—	1(?)†	—	—	—
b	*y sn³ θ/y w*	338	39	22	6	1	8	—	1	—	—	—	1	—	—
				109	34	22	14	2	4	—	—	1(?)	1		
Totals Exp. 2		**1017+**	**187**	**165**			**20**			—		**2(?)**		—	—

* In exp. 1c only head and thorax were inspected. All 31 spots were found on the thorax.

† Doubtful if the single bent seta was genetically *sn³*.

The same conclusion holds true for experiment 2. Here simple segregation should produce two daughter cells of the constitutions *y sn*^{3} *θ* and *y*, giving rise to *sn*^{3} and *y* twin spots. But only two twin spots (one doubtful!) out of 187 were found.

(b) Somatic crossing over at a two strand stage.

Expectation in experiment 1: two daughter cells with the crossover constitutions *y sn*^{3} and *y*; these should result phenotypically in *sn*^{3} and *y* twin spots.

Result: only 2 such twin spots out of 143 spots.

Expectation in experiment 2: two daughter cells with the crossover constitutions *y sn*^{3} and *y θ* giving rise to *y sn*^{3} spots (the *y θ* area not being distinguishable from the surrounding tissue).

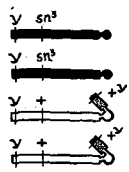
Result: No *y sn*^{3} spot out of 187 spots.

Conclusion: Segregation with crossing over at the two strand stage does not account for the findings.

(c) Somatic crossing over at a four strand stage. This implies for our discussion that only two strands at any one level cross over. Otherwise four strand crossing over would here be indistinguishable from two strand crossing over. Before entering a detailed analysis, it will be shown once more that generally no chromatid reduction occurs in the formation of mosaic areas.

We consider the original constitution of the females in experiment 1 and experiment 2. If reduction occurs in experiment 1 two of the four resulting cells would each obtain a non-crossover strand of the constitution *y sn*^{3}, or *y θ*, and the other two cells would each obtain a crossover strand of the constitution *y sn*^{3} *θ* or *y*. The areas resulting from later divisions of the four reduction products would exhibit the phenotypes *y sn*^{3}, +, *sn*^{3}, and *y*. As the phenotype + is not different from the surrounding not-reduced tissue, the visible result would be a triplet spot with a *y sn*^{3}, *sn*^{3}, and *y* area. No spot of this nature was observed and the *y sn*^{3} phenotype itself appeared not more than three times in 143 spots. As to experiment 2: the four reduction products would have the constitutions *y sn*^{3} *θ*, *y*, *y sn*^{3}, and *y θ*, giving rise to a triplet area with the phenotypes *sn*^{3}, *y*, and *y sn*^{3} as in experiment 1. Again no such spot was found nor a single *y sn*^{3} phenotype out of 187 spots.

Thus there remains the following mechanism to be examined: crossing over between two of the four strands and subsequent segregation two by two of strands without chromatid reduction. The main consequences of this mechanism are diagrammatically represented in figures 8 and 9. At the left of the first horizontal section (a) the four chromatids are represented which originated as a consequence of crossing over in the *sn*^{3}-Theta-attachment region between two of the original chromatids. To the right of this



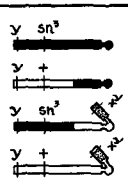
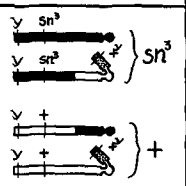
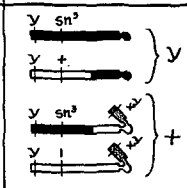
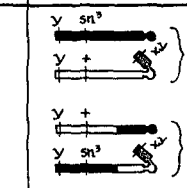
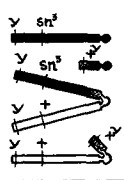
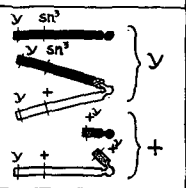
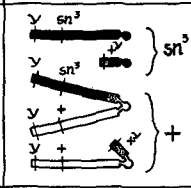
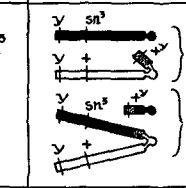
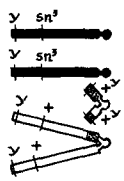
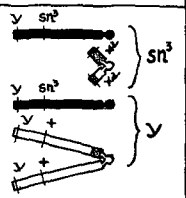
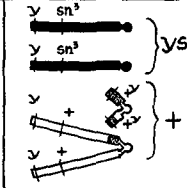
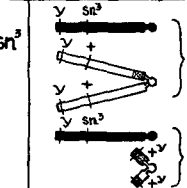
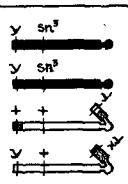
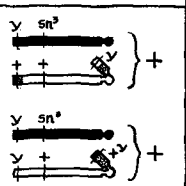
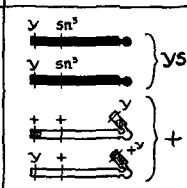
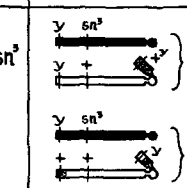
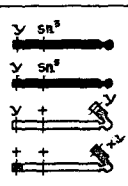
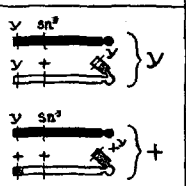
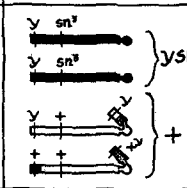
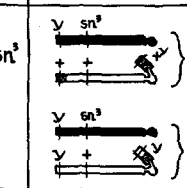
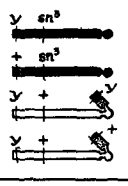
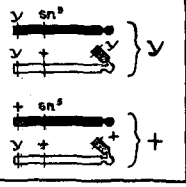
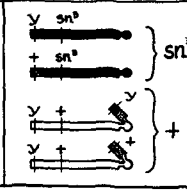
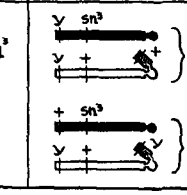
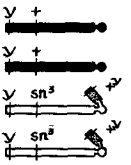
type of cross-over (see text)	resulting chromatids	(X)	(Y)	(Z)
(a)				
(b)				
(c)				
(d)				
(e)				
(f)				

FIGURE 8. *y sn³/yθ.* (See table 13, experiment 1.) Six types of single crossovers and the results of three different types of segregation.



type of cross-over (see text)	resulting chromatids	(x)	(y)	(z)
(a)		+ / sn³	y / +	+ / +
(b)		y / sn³	+ / +	+ / +
(c)		+ / y	y / sn³	y / +
(d)		+ / +	y / sn³	+ / +
(e)		y / +	y / sn³	+ / y
(f)		y / +	+ / sn³	+ / +

FIGURE 9. *y/y sn³θ*. (See table 13, experiment 2.) Six types of single crossovers and the results of three different types of segregation.

four chromatid group are shown the results of the three different possibilities of distributing the chromatids two by two into two daughter cells. In both experiments the first of these possible types, **x**, leads to the appearance of a sn^3 spot, the second, **y**, to that of a *y* spot while the last, **z**, does not give rise in any of the two resulting cells to a phenotype different from the surrounding tissue. If we compare these deductions with the actual results (table 13) it appears that the great majority of spots seem to fit the expectation. In experiment 1, 134 spots out of 143 and in experiment 2, 185 out of 187 spots exhibit phenotypes which would result from the mechanism discussed. The proportion of sn^3 to *y* spots in both experiments is similar also, namely 116:18 in experiment 1, and 165:20 in experiment 2. Before accepting the conclusion that crossing over to the left of the Theta-attachment and an ensuing chromatid distribution according to two different types in a proportion of about 8:1 is responsible for the findings, it will be necessary to see whether there are other types of crossing over and segregation which would lead to the same observed spots. Five further types of crossing over are listed in figures 8 and 9. In the horizontal sections (b) and (c) the possibility is considered that crossing over involves the right (bobbed) end of the Theta fragment and that part homologous to it which is located near the spindle fibre in the X chromosome—(b) in the free X and (c) in the X chromosome to which Theta is attached. In the sections (d)–(f) the possibility is considered that crossing over involves the left (yellow) end of the Theta fragment and the homologous part in the left end of the X chromosomes— (d)between a Theta chromatid and the chromatid of the X chromosome to which the Theta chromatid is attached, (e) between a Theta chromatid and the sister X chromatid of that to which the Theta chromatid is attached, and (f) between a Theta chromatid and a chromatid of the free X chromosome. If somatic crossing over obeys the same rule as does germinal crossing over, then it can be predicted that the crossover processes represented in sections (b)–(f) will play only a very small role, if any, in comparison to the crossover type referred to in (a). This follows from the generally established fact that short duplications take very little part in germinal crossing over (DOBZHANSKY 1934). In addition there may be quoted the results of unpublished experiments on germinal crossing over in the X chromosomes of females possessing one Theta attachment: Only 2 out of 7559 individuals occurred as a result of crossing over between Theta and the homologous region near the spindle fibre of an X chromosome. But in spite of the rareness of such crossing over types during germ cell formation, we have to consider the consequences of their possible occurrence in somatic tissues if followed by segregation. For, lacking any previous acquaintance with

somatic crossing over, we are not justified at this stage to apply our knowledge of germinal processes to somatic ones.

The procedure will be to tabulate which types of crossing over and segregation are able to give rise to the two main kinds of spots which were observed, namely *sn*^{3} and *y*.

It is seen (fig. 8) that *sn*^{3} spots originate in experiment 1 only according to the types **ax, by,** and **fy.** In experiment 2 (fig. 9) *sn*^{3} spots can originate according to types **ax** and **fy.** Other types in which *sn*^{3} appears as part of a twin segregate will not be considered for the moment.

The process **by** in experiment 2 does not give rise to *sn*^{3} spots. It therefore seems sufficient to consider only **ax** and **fy** as possibly responsible for the observed *sn*^{3} spots. Now the process **fy** should hardly occur without the occurrence of **dy** and **ey,** for a striking preference of the Theta fragment for crossing over with different kinds of the distant yellow ends of X chromosomes is not likely. However, **dy** and **ey** give rise to *y sn*^{3} spots in experiment 1 and to *y* and *sn*^{3} twin spots in experiment 2. Such spots were negligible in number—and they can also originate through different processes. It is therefore concluded that no appreciable percentage of **fy** processes occurred and that the majority of *the sn*^{3} *spots both in experiment 1 and 2 were produced as results of the process* **ax.**

Yellow spots would appear as a result of the following processes in experiment 1: **ay, bx, ex, ez,** and **fx.** In experiment 2 yellow spots will result from **ay, cx, cz, ex, ez,** and **fx.** The processes **ey, ex, ez,** and **fx** agree in both experiments in yielding only *y* spots. On the other hand, **bx** would be responsible for *y* and *sn*^{3} twin spots in experiment 2, and **cx** for *y* and *sn*^{3} twin spots in experiment 1. But twin spots have to be considered also, as some of them will appear phenotypically only as *y* areas (others as *sn*^{3} areas). Besides **bx, cx,** and **cz** we have, therefore, to discuss still the processes **cy, by,** and **ey,** which give rise to *y* and *sn*^{3} twin spots in experiment 2. The last named group of processes, if they occur, gives rise to *y sn*^{3} spots in experiment 1. Actually only 2 *y sn*^{3} spots out of 143 were found in experiment 1 so that these processes at best can account only for a small number of *y* spots in experiment 2 and cannot account at all for any of the 18 *y* spots in experiment 1. We are then restricted to an analysis of the types **ay, bx, cx, cz, ex, ez,** and **fx.** Do all these crossing over and segregation processes occur? A decision cannot be reached without taking recourse to other data. We have seen in the preceding chapter on spots in *Mn* or Bld-bearing females that the occurrence of types of segregation which are reductional with respect to sister fibre attachment points could be excluded. If we use this knowledge for our present problem we see that the process **ay** representing such a reductional segregation becomes eliminated

and only equational segregation processes remain. They all have in common the fact that they involve crossing over between the Theta duplication and the X chromosome, **bx, cx** and **cz** crossing over in the homologous right regions and **ex, ez** and **fx** in the homologous left regions. Whether both kinds of crossovers contribute to the *y* spots or if only one kind occurs and if so, which one it is, cannot be ascertained from the present experiments. But further information will be yielded in an analysis of experiments which involve *y*, *sn*^{3}, *Mn*, and *θ*. No discussion will be devoted to the *y sn*^{3} spots nor to the *sn*^{3} and *y sn*^{3} twin spot. Simple elimination, non-disjunction processes, or multiple crossovers at one or successive stages may be involved.

The results of this chapter can be summarized as follows:

(1) Somatic segregation is always (or nearly always?) preceded by somatic crossing over between two of four chromatids.

(2) If the result of a preceding section is held to be generally true, namely, that segregation is always equational with respect to spindle fibre attachments, then it is found that somatic crossing over involves not infrequently the Theta-duplication which only very rarely participates in germinal crossing over.

On the basis of these findings it is postulated that somatic crossing over underlies segregation also in those cases where the genetic constitution, on account of the absence of an attached Theta duplication, does not seem to allow a direct test of crossing over in the rightmost region. Such a test, however, is possible under some circumstances as will be shown in the following section.

Experiments involving bobbed as a means of determining the rightmost crossover region

As pointed out before, the fact had been unexpected that in flies heterozygous for bobbed (*bb*) or one of its alleles "elimination" of the not-bobbed X chromosome generally does not result in spots exhibiting the bobbed phenotype. Table 14, experiments 2 and 3, contains data on such spots in flies which had *Mn* in one X and *bb* or *bb*^{l} (a lethal allele of *bb*) in the other one. It is seen that 34 of the spots in experiment 2 possessed phenotypically non-*bb* setae, and that in experiment 3 where only non-*bb* spots would have been visible, 10 spots were found. Leaving aside at present the spots with smaller than normal setae, the non-appearance of the *bb* character in these spots has to be explained.

The assumption of non-autonomous development of the bobbed character from cells of a mosaic area, although possible, seemed unlikely, especially in view of the result of an extensive test of the sex-linked factor "tiny bristle" (*ty*, 1, 44.5) which is very similar in action to bobbed, to-

TABLE 14

Seta length in spots of individuals containing bb and bb^1 (exp. 2 and 3). Experiment 1 is the control.

EXP.	CONSTITUTION	INDIVIDUALS	SPOTS	TYPE OF SPOT (LENGTH OF SETAE) +	<+
1	$\frac{+\ g^2\ Mn}{y\ +\ +}$	528	58	54	4†
2	$\frac{+\ g^2\ Mn\ +}{y\ +\ +\ bb}$	769	45	34	11*
3	$\frac{+\ g^2\ Mn\ +}{w^e\ +\ +\ bb^1}$	476	10	10	?

† Only spots are recorded which enclose macrochaetae on head or thorax. They were *y* in exp. 1 and 2 and $+^M$ in exp. 3. In the latter experiment spots with setae shorter than $+^M$ could not be distinguished from the rest of the body.

‡ Setae length as in *bb* ♀ : 2 spots.
Setae length >"*bb* ♀," but <+ : 2 spots.

* Setae length as in *bb* ♀ : 4 spots.
Setae length >"*bb* ♀" but <+ : 6 spots.
Setae length like *bbXO*♂ : 1.

gether with which it can be classified as a recessive Minute. The test consisted in observing whether *ty* behaved autonomously in spots or not. The result was that it did, which made the discordant behavior of *bb* more surprising.

The theory of somatic crossing over in the form proposed allows us to understand the apparently exceptional behavior of *bb* if we make a specifying assumption concerning the region of somatic crossing over in the X. It has been pointed out before that in the majority of cases this region lies between the locus of *Mn* and the right end of the X. If the point of crossing over were located between the rightmost factor, which is bobbed, and the right end of the X, then the behavior of the phenotype bobbed in spots would be exceptional also under the somatic crossing over theory, for the equational segregation of fibre attachment points is by necessity reductional for all loci to the left of the crossover point. Therefore one of the segregation products of a fly which is heterozygous for bobbed becomes homozygous for this locus. Thus in a *Mn/y bb* female crossing over at the four strand stage, if occurring to the right of *bb*, would lead to daughter cells of the constitutions *Mn/Mn* and *y bb/y bb*, the latter giving rise to *y* spots. These spots, in spite of their assumed homozygous condition for *bb*, show long not-*bb* bristles. Or let us consider experiment 4 of table 5. In females of the constitution *y . . . bb^1/sn^3bb^x*, four strand crossing over

to the right of the *bb* alleles should yield daughter cells of the constitutions *y . . . bb^{l}/y . . . bb^{l}* and *sn^{3}bb^{z}/sn^{3}bb^{z}*. From the knowledge of the recessive lethal action of *bb^{l}* in zygotes the most likely assumption would be a cell lethal action which hinders the *y . . . bb^{l}/y . . . bb^{l}* cell from developing into a spot area. However, two *y* areas in *y-sn^{3}* twins were found covering 3 setae and four covering 2 setae besides numerous one-seta *y* areas (tables 7 and 5). The difficulties disappear if we assume that the point of somatic crossing over lies to the left of the *bb* locus. This assumption agrees with the fact that as a rule no *germinal* crossing over occurs between *bb* and the fibre locus (STERN 1929). Crossing over to the left of *bb* will leave the constitution of the daughter cells unchanged with regard to *bb*. In *Mn/y bb* flies the daughter cells will be *Mn +/Mn bb* and *y +/y bb* and the resulting *y* spot, heterozygous for *bb* like the rest of the fly, will have not-*bb* bristles; in *y . . . bb^{l}/sn^{3}bb^{z}* females the twin spots will have the constitutions *y . . . bb^{l}/y . . . bb^{z}* and *sn^{3}bb^{l}/sn^{3}bb^{z}*, again not differing from the surrounding tissue in regard to *bb*.

There remains to be given an explanation of the 11 spots in experiment 2 which exhibited *y* setae of shorter than normal length. That they are not due to a special behavior in regard to *bb* is probable from the control experiment 1 in which *bb* was not involved at all and in which 4 spots with shorter setae were found. These 4 spots are to be expected as results of crossovers between *y* and *Mn* thus producing spots of the constitution *y Mn/y +*, the variation in setae length representing the known variability of *Mn* setae in the presence of different modifiers (which in these spots must be of varying combinations due to different crossovers). The same explanation is adopted for 10 of the 11 spots with smaller than normal setae in experiment 2, while the single bristle spot with a very short, *bb* XO-like seta was perhaps due to a real elimination of the *Mn* X chromosome. The higher frequency of the smaller spots in 2 than in 1 is within the limits of variability of somatic crossing over (the difference also being smaller than three times the standard error). It might be added that experiments 2 and 3 were conducted in such a way as to exclude the presence of a supernumerary Y chromosome which would have covered the *bb* effect.

The assumption that the crossover point lies always to the left of *bb*, can be applied to an independent test of the origin of the *y* spots which appear in the Theta experiments discussed in the preceding section. We had seen that the *y* spots may have resulted both from a reductional segregation process after crossing over between the X chromosomes (**ay** process) and from equational segregation processes after crossing over involving the Theta duplication (processes **bx . . . fx, cz, ez**), The first alternative could

not be rejected on the basis of an analysis of the pertinent data but only on the basis of evidence from earlier experiments.

If the *y* spots occurred in consequence of processes **ay,** after reductional distribution of the fibre points, then a reductional distribution for the *bb* locus would have occurred also. Bobbed then should exhibit its phenotype in suitable spots. But if the *y* spots were due to any of the processes **bx . . . fx, cz, ez,** then *bb* should not appear phenotypically. This point could have been tested in experiments 1b and 1c of table 13. In experiment 1b five *y* spots occurred. A reliable distinction of *bb* and not-*bb* setae generally can be made only in macrochaeta of the head and thorax and in flies which do not carry a Minute factor. Only 3 of the 5 *y* spots occurred in non-Minute flies and none included head or thorax macrochaetae. Experiment 1c represents a special attempt to obtain *y* spots covering suitable setae. Of the nearly 3000 individuals inspected only 18 possessed *y* head-thorax spots, 4 of which covered macrochaetae. Three of these spots had setae which were clearly of about normal, not bobbed size; one had setae of a size similar to *bb*. This last named *y* spot seems to indicate the occurrence of the **ay** process. However, too much weight should not be laid on this single finding which is contradicted by sufficient other evidence. It could, for instance, be regarded as a case of crossing over to the right of *bb* with normal disjunction of the fibre points. However, this is not suggested as a probable explanation.

The other three not-bobbed, yellow spots establish definitely the occurrence of at least some of the crossing over and segregation processes **bx . . . fx, cz, ez.** It is probable that one or both of the processes **b** and **c** were involved, as the bristle size in these spots, though not-bobbed, was apparently somewhat abnormal. This points to a 3X (superfemale) constitution of the spots, as to be expected from **bx** and **cx** or **cz** (fig. 8).

The number of X chromosomes in cells of spots

If mosaic spots in females which do not contain a duplication originate by segregation of four chromatids two by two following crossing over between two X chromosome chromatids, then the X chromosomes in cells constituting the mosaic regions must be present in the diploid number. This expectation is in contrast to that derived from a simple elimination hypothesis which leads to the expectation of finding one X chromosome only. Still other numbers of chromosomes can be predicted after segregation in females carrying a duplication as can be seen in figures 8 and 9. An investigation of the number of X chromosomes in mosaic spots should provide new tests for the proposed theories.

It was not thought possible to determine the number of X chromosomes

by a cytological study as no cell divisions are expected in the hypodermal cells which constitute the spots of adult individuals. Genetic means, however, are available. BRIDGES (1925) used two criteria: the presence (♂ = X) or absence (♀ = XX) of a sex comb in case of a spot covering the sex comb region of a front leg and the shade of w^e (eosin 1, 1.5) color in eye spots of individuals which according to their genetic constitution exhibited eosin in a mosaic eye part. Eosin is known to be pink in females and pinkish yellow in males. Since the sex comb region is involved only very rarely in a spot and since the eosin test allows for reliable judgments of the eye color only in comparatively large spots, a third criterion for X or XX condition was used primarily in the present studies, namely, the coloration of the fifth and sixth abdominal segment.[1] These segments are dark in males and, in most stocks, colored only along the posterior margins and near the median line in females. This coloration is cell-autonomous as many cases ranging from smallest to largest black spots on female segments in certain experiments show. A fourth criterion of the X or XX constitution of spots can be derived from a consideration of bobbed spots as will be shown in the next section.

The determination of the sex of spots as an index of the number of X chromosomes was made in all later experiments after the importance of this condition was recognized. In the earlier experiments the determination of sex was not made while the flies were alive. However, it seems certain to me that I would have recorded the fact of male coloration in abdominal spots had it occurred, as I wrote down individual records for each spot noting the numbers of bristles involved, the abdominal segment, and often added a sketch of the spot. Whenever no comment was made in my notes (in 22 out of 147 cases) it can be regarded as highly indicative of the absence of male coloration, that is, of the presence of two X chromosomes.

Consequently all those spots found in the experiments discussed in the preceding sections of this paper in which two or more setae of the critical abdominal region were involved have been tabulated in table 15. Besides, the results of three further experiments reported elsewhere (STERN 1935b) have been added. It will be noted that the mosaics have not been classified under the alternative "female color or male color" but rather under "male color or no male color." The latter is better, since it is possible that in rare cases very narrow spots do not exhibit male coloration in spite of 1X constitution even if they cover two or more setae. The table shows that the great majority of mosaics, 132 out of 147, did not exhibit male coloration. They thus contain cells with the constitution XX, in agreement with the

[1] Owing to the fact that the apparent first segment is regarded to be originally composed of two segments, the morphologically fifth and sixth segments are the ones which appear as fourth and fifth without knowledge of the homologies.

theory of crossing over and segregation and in contradiction to the elimination theory. Fifteen spots exhibited male coloration, an effect of one X constitution.

The probable origin of most of these exceptional spots will be discussed later in connection with the influence of an extra Y chromosome on somatic crossing over. It is significant that no male color appeared in any of the 31 spots which exhibited the typical result of segregation, the twin condition. Here it is pointed out only that the male-colored spots occurred in a few very nearly related cultures in each experiment: 5 of the 6 male spots in line 1 of table 15 came from 3 related cultures of a total of 34, all 3 male

TABLE 15

The sexual coloration in critical abdominal spots. Numbers in ( ) represent those of the total number of spots which were recorded without a comment as to sexual coloration.

EXPERIMENTS	MAIN FACTORS INVOLVED	sn^3		y		$y\ sn^3$		y-sn^3		+ ♂ COL.	NO ♂ COL : ♂ COL.
		NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.		
Table 5, 2–4†	y, sn^3	7(2)	3	7(3)	3	—	—	22(1)	—	—	36:6
Table 8, 1–4	y, sn^3	—	—	9	—	13	1	—	—	2	22:3
Table 10, 1–4‡	y, sn^3, Mn	—	—	2	—	7	—	—	—	2	9:2
Table 13, 1a, b	y, sn^3, θ	12(3)	—	1(1)	—	—	1	—	—	2	13:3
Table 13, 2a*	y, sn^3, θ	9(9)	—	4(3)	—	—	—	—	—	—	13:0
$y\ Hw/sn^3$		1	1(?)	—	—	—	—	9	—	—	10:1(?)
$y\ Hw$ dl-49/+		—	—	16	—	—	—	—	—	—	16:0
$(y\ sc)^-/sn^3$		13	—	—	—	—	—	—	—	—	13:0
										Total:	132(22):15(?)

† No abdominal spots were recorded in exp. 1.

‡ No critical spots occurred in exp. 3; for exp. 2 see text.

* No critical spots occurred in exp. 2b.

spots in line 2 from one single culture of a total of 29 and both male spots in line 3 from one single culture of a total of 32. This indicates that special genetic conditions were responsible.

The two normal, male-colored spots in line 4 will be commented upon later.

A comprehensive experiment involving y, sn^3, Mn and Theta

Each of the experiments dealt with in the preceding chapters yielded results which, taken together, furnish a consistent picture. However, some points were cleared up only by referring from one experiment to another and other points were left completely unsolved. The present chapter gives an account of experiments in which *y*, sn^3, *Mn*, and θ were involved at the same time. Emphasis will be laid on two questions: (1) Are the *y* spots

TABLE 16a

Spots in experiments involving primarily y, sn^3, Mn, and θ.

EXP.	CONSTITUTION	IND.	SPOTS	FREQUENCY IN % OF ALL SPOTS	% y SPOTS	y 1	y 2	y >2	sn^3 1	sn^3 2	sn^3 >2	+ 1	+ >2	+ ♂ COL.	y sn^3 1	y sn^3 >2	y-sn^3 2	y-sn^3 >2	OTHER SPOTS
1a	$\frac{y + + Mn +^{bb}}{y\ w^e\ sn^3 + \theta}$	219	82	38	7	2	1	12	19	3	42	—	1	—	—	—	—	2	—
b	$\frac{y + + Mn\ bb}{y\ w^e\ sn^3 + \theta}$	1384	285	21	5	30	5	32	93	26	91	—	—	—	—	—	1	5	2*
c	$\frac{y + + Mn\ bb'}{y\ w^e\ sn^3 + \theta}$	221+	59	27	10	8	1	14	15	8	10	1	—	—	—	1	—	1	—
	Totals Exp. 1				**5.8**		**105**			**307**			**2**	—		**1**		**9**	**2**
2a	$\frac{y\ w^e\ sn^3\ Mn +^{bb}}{y + + + \theta}$	97	6	6	5	3	—	2	—	—	—	—	—	1	—	—	—	—	—
b	$\frac{y\ w^e\ sn^3\ Mn\ bb}{y + + + \theta}$	260	13	5	3	2	1	6	1	—	—	1	2	—	—	—	—	—	—
c	$\frac{y\ w^e\ sn^3\ Mn\ bb'}{y + + + \theta}$	139	19	14	10	8	2	4	1	1	1	2	—	—	—	—	—	—	—
	Totals Exp. 2				**5.6**		**28**			**4**			**5**	**1**	—	—	—	—	—
3a	$\frac{y + + Mn\ \theta}{y\ w^e\ sn^3 + +^{bb}}$	857+	163	19	4	22	3	5	64	25	40	—	—	—	1	1	—	2	—
b	$\frac{y + + Mn\ \theta}{y\ w^e\ sn^3 + bb}$	95	16	17	2	2	—	—	3	3	8	—	—	—	—	—	—	—	—
	Totals Exp. 3				**3.4**		**32**			**143**		—	—	—		**2**		**2**	—
4a	$\frac{y\ w^e\ sn^3\ Mn\ \theta}{y + + + +^{bb}}$	557	41	7	5	16	7	4	5	2	4	1	—	2	—	—	—	—	—
b	$\frac{y\ w^e\ sn^3\ Mn\ \theta}{y + + + bb}$	348	29	8	4	12	—	3	2	3	3	—	1	5	—	—	—	—	—
c	$\frac{y\ w^e\ sn^3\ Mn\ \theta}{y + + + bb'}$	221	19	9	5	4	4	2	4	1	2	—	—	2	—	—	—	—	—
	Totals Exp. 4				**4.6**		**52**			**56**			**2**	**9**	—	—	—	—	—
	Totals Exp. 1–4	**4398+**	**732**																

* 1 spot showed thick heavy bristles of *M* length, the other was a sn^3 (very small seta) and sn^3 twin spot.

which occur in experiments involving Theta really results of crossing over between Theta and the X chromosome instead of results of crossing over between the X chromosomes and subsequent reductional separation of sister spindle points? (2) Do both the left and the right region of the Theta duplication cross over with their homologous regions in the X chromosome?

Four different combinations of *y*, *sn*^{3}, *Mn*, and Theta were investigated (table 16a). In all experiments eosin was involved also and in each of the four combinations different sub-groups were present with either the normal allele of bobbed, or two mutant alleles similar to each other, *bb* or *bb'*. (*bb'* has not as yet been described). No consistent differences between the

TABLE 16b

Spots (from table 16a) which could be classified according to seta length.

EXP.	*y*+^{M}		*y M*		*sn*^{3}+^{M}		*sn*^{3} *M*		*y-sn*^{3}
	1	>1	1	>1	1	>1	1	>1	>
1	10	20	3	1	30	92	10	—	3*
2	—	6	—	1	1	2	—	—	—
3	1	1	—	—	8	22	8	—	2†
4	1	2	1	—	—	—	1	—	—

* *y*+^{M}–*sn*^{3}+^{M}: 1 spot.
y+^{M}–length of *sn*^{3} not determinable: 1 spot.
y M(?)–length of *sn*^{3} not determinable: 1 spot.
† *sn*^{3}+^{M}–length of *y* not determinable: 2 spots.

sub-groups are apparent. The frequencies of total number of spots per hundred individuals varied between 5 and 38, but a consistent grouping is clear, giving the highest frequencies to experiment 1, the next to experiment 3, while experiments 2 and 4 present the lowest figures. Striking differences between the relative frequencies of the different types of spots, especially the *sn*^{3} and *y* spots are also obvious. While in table 16a all *y* spots and all *sn*^{3} spots were listed together regardless of their length, table 16b shows that among both groups there were spots with bristles of about normal length as well as of Minute length. An accurate determination of length in spots occurring on the abdomen or including only microchaetae was not possible in general, so that the majority of spots could only be classified in regard to *y* or *sn*^{3}. However, table 16b indicates that the great majority of spots were not Minute. Furthermore all 19 *sn*^{3} Minute spots were single bristle spots so that it seems probable that all or most of the larger *sn*^{3} spots were not Minute, and even among the single bristle spots the majority of the head-thorax spots were not Minute. This holds primarily for experiments 1 and 2. The bristles classified as not-Minute were

TABLE 17

Spots resulting from **x** *(equational) and* **y** *(reductional) segregation in experiments involving y, sn^3, Mn and θ.*

CROSSOVER REGION	EXP. 1 $\frac{y + Mn}{y\ sn^3 + \theta}$ (x)	(y)	EXP. 2 $\frac{y\ sn^3\ Mn}{y + + \theta}$ (x)	(y)	EXP. 3 $\frac{y + Mn\ \theta}{y\ sn^3 +}$ (x)	(y)	EXP. 4 $\frac{y\ sn^3\ Mn\ \theta}{y + +}$ (x)	(y)
(a) sn^3-Mn	$sn^3\ Mn$	+	$sn^3\ Mn$	+	$sn^3\ Mn$	y	$sn^3\ Mn$	y
(b) Mn-θ	sn^3	$y\ Mn$	+	$y\ Mn$	sn^3	$y\ Mn$	+	$y\ Mn$
(c) right end of θ and of X chromosome bearing θ	y(3X) dies		y(3X) dies		$y(M/M/+)$ $sn^3/\underline{\theta\theta}$		$y(M/M/+)$ $sn^3/\underline{\theta\theta}$	
(d) right end of θ and of free X chromosome	$y(M/M/+)$ $sn^3/\underline{\theta\theta}$	dies +(3X)	$y(M/M/+)$ $+/\underline{\theta\theta}$	dies +(3X)	y(3X) dies	$y(M/M/+)$ $sn^3/\underline{\theta\theta}$	y(3X) dies	$y(M/M/+)$ $+/\underline{\theta\theta}$
(e) left end of θ and of X chromosome (3 possibilities)	$y\ Mn$		$y\ Mn$		$y\ Mn$		$y\ Mn$	
(f)	—		—		—		—	
(g)	$y\ Mn$		$y\ Mn$		$y\ Mn$		$y\ Mn$	

often found to be somewhat abnormal, slightly thickened or shortened in varying fashion. The causes for this will soon become clear.

If one tabulates for all four experiments the expectations for spots derived after different types of crossing over and segregation, a relatively simple explanation of the results appears. An abbreviated list of expectations is presented as table 17. Only single crossovers are considered and only the **x** and **y** type of segregation, the first being equational for the fibre points, the second reductional. The **z** type which is also equational does not lead to visible spots after single crossing over between the X chromosomes.

Process **a.** As the table indicates, crossing over between sn^3 and *Mn* with normal disjunction of the fibre points **ax** leads to sn^3 *Mn* spots in all four experiments. Non-disjunction of the fibre points **ay** leads to normal bristle spots in experiments 1 and 2 and to *y* not-*Mn* spots in experiments 3 and 4. No other types of crossing over and segregation represented in table 17 besides **ax** result in sn^3 *Mn* spots. These spots, in all four experiments, are therefore regarded as produced by the process **ax** (the actual non-occurrence in experiment 2 is probably due to the comparatively small number of flies inspected). In good agreement with the findings in the earlier experiments involving *y*, sn^3, and *Mn* is the fact that somatic crossing over to the left of *Mn* yields only single seta spots. This is interpreted as before to mean that crossing over away from the fibre region occurs only late in development.

The occurrence of the process **ay** can at most account for the + spots in experiments 1 and 2 and for the *y* spots in experiments 3 and 4. However it is more probable that these spots owe their existence to different processes to be discussed below.

Process **b.** Crossing over between *Mn* and the end of the X and equational segregation for the fibre points **(bx)** leads to sn^3 not-*Mn* spots in experiments 1 and 3, to normal bristle spots in experiments 2 and 4.

Spots which are sn^3+ may be expected also from some of the processes **c** and **d.** It is seen that segregation products in **c** and **d** will contain one X chromosome and two Theta duplications. Whole individuals of this constitution are not viable (unpublished results). In case hypodermal areas of this constitution are able to survive one would expect to find male sex indications in suitable spots. Actually, as will be shown soon, the great majority of sn^3+^M spots did not exhibit male characteristics. This leads us to the conclusion that most of the sn^3+^M spots in experiments 1 and 3 owe their origin to the process **bx.** One would expect a high corresponding number of normal bristle spots in experiments 2 and 4. Only few such spots were found although their relative frequencies in experiments 2 and 4 are higher than in experiments 1 and 3. However, on account of the great

variability in the occurrence of larger sized spots in the head-thorax region in different experiments no serious obstacle is seen in this lack of normal spots which are only visible under the conditions just stated. And it is significant in this respect that of the 100 *y* or sn^3 spots in experiments 2 and 4 only 15 could be classified in regard to seta length. While this type of crossing over to the right of *Mn* with normal segregation can account for the majority of sn^3 not-*Mn* spots, non disjunctional segregation of the fibre points **by** can at most account only for the small number of *y Mn* spots and other processes will also yield this type of spot.

Processes **c, d.** An explanation for the great number of $y+^M$ spots and their varying percentage in the different experiments is provided by the assumption of crossing over between the right end of an X chromosome and the homologous part of the Theta duplication. Two main types of crossovers are possible and assumed to occur with equal frequencies: process **c,** crossing over between Theta and an X chromatid with the Theta duplication; and process **d,** crossing over between Theta and a free X chromatid.

It is seen that normal disjunction **(x)** of the fibre points after crossing over **c** in experiments 1 and 2 yields triple-X *y* spots which possess *Mn* in one of the three X chromosomes. Such a constitution $M/+/+$ is known to produce not-Minute bristles, while the twin segregate whose single X chromosome contains *Mn* is inviable. In experiments 3 and 4 crossing over **c** and normal segregation leads to sister cells, one with 3 X chromosomes and one with one X chromosome and two Theta duplications. The cell with three X chromosomes carries two *Mn* factors. As individuals of such constitution as well as individuals with one X and two duplications are not viable it is assumed that hypodermal segregation products of these constitutions will at best be of low viability and only rarely give rise to spots. No reductional segregation for the spindle fibre points is expected to occur after crossing over of type **c,** as this would mean non-disjunction of the two complete non-crossover sister chromatids which do not carry Theta, a process which throughout this paper has been shown either not to occur at all or at best as an extremely rare exception.

Crossing over **d** with equational segregation **(x)** in experiments 1 and 2 leads to the similar types of inviable or barely viable twin cells with triplo-X $Mn/Mn/+$ and $X+^M$ cells with two Theta duplications, but in experiments 3 and 4 to triplo-X yellow $Mn/+/+$ cells and inviable X *Mn* partners.

Reductional segregation **(y)** leads to triplo-X cells with normal appearing bristles in experiments 1 and 2 and to combinations with low viability in experiments 3 and 4. Spots with these phenotypes are of course to be expected also from other processes.

Altogether equational segregation after crossing over **c** leads to *y* spots in experiments 1 and 2 and after crossing over **d** to *y* spots in experiments 3 and 4. The triplo-X *Mn*/+/+ constitution of these spots agrees with the somewhat abnormal appearance of the *y*-bristles, the triplo-X condition making for heavier than normal bristles, the one *Mn* possibly accounting for the slight decrease in bristle length.

The frequencies of *y* spots in experiments 1 and 2 should be higher than in experiments 3 and 4 depending on the degree of viability of triplo-X *Mn*/*Mn*/+ cells. If this viability is zero, the percentage of *y* spots in experiments 1 and 2 would be twice that in experiments 3 and 4, for one of the two normal disjunctional types of segregation, **(z),** does not lead to visible spots in experiments 3 and 4 while it is identical in effect with **x**-segregation in experiments 1 and 2. The actual average frequencies are 5.7 per cent for experiments 1 and 2 and 4.0 per cent for experiments 2 and 3. (These figures are based on total *y* spots and are not corrected for the presence of *y Mn* spots.) Calculation shows that such a difference would follow if the chance of finding a *y Mn*/*y Mn*/*y* spot is about one-third of that of finding a *y Mn*/*y*/*y* spot. While this result points to a definite viability of triplo-X spots with two *Mn* factors, not too much weight should be attached to the value obtained.

Processes **e–g.** Finally we have to consider the possibility of crossing over between the left end of Theta and the homologous left end of the X chromosomes. Assuming only equational fibre segregation, two of these processes, **e** and **g,** can lead to the production of *y Mn* spots, while process **f** will not yield visible spots. This represents an alternative to the reductional process which also can account for *y Mn* spots. As the occurrence of reductional somatic segregation has been excluded or made improbable in other cases, it is assumed that the *y Mn* spots are products of equational segregation following the processes **e** and **g.**

Summarizing, we can state that equational segregation of fibre points after single crossing over between the X chromosomes in the region from *Mn* to fibre point and crossing over between the right end of an X chromatid and Theta with equational segregation can account for the great majority of spots, namely the sn^3+^M and $y+^M$ spots. Crossing over with equational segregation occurs also to the left of *Mn*, at a low frequency, as witnessed by the sn^3Mn spots which are products of crossing over in the sn^3 to *Mn* region; proof of crossing over in the w^e to sn^3 region is seen in the occurrence of one out of four sn^3+^M spots which all enclosed eye or ocellar areas but of which the one did not show eosin eye color.

Crossing over between the homologous left regions of the X chromosome and the Theta duplication with equational fibre segregation is assumed to occur and to account for the *y Mn* spots. No detailed attempt will be made

to explain the origin of the remaining classes of spots, which all have few representatives only. Multiple crossing over and occasional new crossover processes in descendants of crossover cells will account for them as it will also for a fraction of the main classes.

The high frequency of crossing over between Theta and the homologous right end of the X chromosomes is surprising in view of the rareness of this process in germinal crossing over. In order to get an estimate, however inaccurate, of this frequency the data of experiment 1 may be considered. The frequencies of *y* spots and of sn^3 spots are 105 and 307. A certain percentage of the *y* and of the sn^3 spots is *Mn*. If the figures of table 16b are taken as a basis for estimating the $y+^M$ and $sn+^M$ spots—a procedure which is doubtful if viewed in the light of our findings concerning the different ontogenetic time at which different crossover processes occur, we find $(30 \div 34)105 = 93$ *y*+ spots and $(122 \div 132)307 = 284$ sn^3+^M spots or about 1 *y*+ : 3 sn^3+. This means that out of each four crossovers in the right end regions one involves the Theta duplication. This is considerably higher than the relative frequency of Theta crossovers in similar experiments without the presence of *Mn* (table 13). However it is still far from what would result from chance crossing over within a multivalent between the four right ends of the X chromosomes and the two Theta duplications. Excluding sister strand crossing over there would be the following types of crossing over: 4 cases of "X with X"; 4 cases of "free X with Theta"; 2 of "duplication-X with the one Theta duplication"; 2 of "duplication-X with the sister Theta duplication." Assuming normal disjunction of fibre points in 50 per cent of the first group, sn^3+^M spots would result. No visible spots would occur in consequence of crossovers according to the second and third group, but in all cases of the last group $y+^M$ spots would be produced. Chance pairing thus would lead to a 1:1 proportion of $y : sn^3$ spots instead of the observed 1:3 relation.

It is probably significant that crossing over involving Theta is rare in germinal cells where there is a high frequency of crossovers away from the attachment region, while such crossing over occurs frequently in somatic crossovers of flies without *Mn* (table 13) where crossing over becomes concentrated in the proximal region. It is significant that Theta crossing over is relatively still more frequent in the present experiments where, under the influence of *Mn*, the shifting of crossing over to the attachment region has been increased.

As judged by the rareness of *y Mn* spots the frequency of crossing over involving the left end of the Theta duplication is considerably lower than that involving the right end of the duplication.

In table 18 the information as to the sex of spots in this series of experiments is presented. At least 21 out of 25 sn^3 spots were female, as was to

be expected after crossing over between the X chromosomes. All three critical *y* spots were probably female in constitution again as expected, as was one w^e-colored eye-spot which did not affect any setae. Besides these female spots, however, 4 sn^3 male spots and 10 male-colored spots with normal setae were found. It seems probable that these spots represent occasional survivals and division products of 1X cells with two Theta duplications produced from crossing over between the right end of an X chromosome and Theta. The same explanation applies to at least two of the three male-colored spots recorded in line 4 of table 15.

TABLE 18

The sexual coloration in critical spots from experiments involving y, sn^3 Mn, and θ (see tables 13, 17).

EXP.	sn^3 (ABDOMINAL)		w^e sn^3		+	w^e	*y*	
	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	♂ COL.	♀ COL.	NO ♂ COL.	♂ COL.
1a	3	—	—	1	—	—	—	—
b	15	2	—	1	—	—	—	—
c	1	—	—	—	—	—	—	—
2a	—	—	—	—	1	—	—	—
3a	2	—	—	—	—	1*	—	—
4a	—	—	—	—	2	—	—	—
b	—	—	—	—	5	—	1(?)	—
c	—	—	—	—	2	—	2(1?)	—
Total	**21 ♀**	**2♂**	—	**2**	**10**	**1**	**3**	—

* No setae affected.

It is in agreement with this assumption that the sn^3 male spots are not found in experiments 2 and 4 where they are not expected and that likewise the + male spots are not found in experiments 1 and 3 where they should not occur. The very small numbers of critical spots in experiments 2 and 3 diminish somewhat the weight of these facts.

The analysis of this group of experiments furnishes independent evidence of:

(1) the occurrence of equational segregation of the fibre points as the main, if not the only type;

(2) the frequent occurrence of somatic crossing over between the homologous right regions of the Theta duplication and the X chromosomes;

(3) the lower frequency of somatic crossing over between the homologous left regions of the Theta duplication and the X chromosomes and

(4) the low frequency of crossing over away from the fibre point region in experiments involving *Mn*.

Points (2) and (4) are probably connected causally.

AUTOSOMAL MOSAICS

Somatic autosomal crossing over and segregation

As has been referred to earlier, spots affecting autosomal characters have been found to occur in flies which carry autosomal Minutes. In a preliminary communication (STERN 1927b) an explanation for these spots was offered which was modeled after the hypothesis of chromosome elimination. A reinterpretation is now necessary in the light of our present knowledge regarding somatic segregation. The experiments to be discussed concern mosaic spots which have occurred in flies heterozygous for the third chromosome dominant Minutes *My*, *Mw*, and *Mβ*. The spots were recognized as areas exhibiting the phenotype of recessive alleles which were present in the zygote in the heterozygous state only. No third chromosome

TABLE 19

Frequencies of autosomal spots in experiments with My, Mw, Mβ, and M33j.

CONSTITUTION	*M*-INDIVIDUALS ♀	*M*-INDIVIDUALS ♂	SPOTS ♀	SPOTS ♂	SPOTS SEX?	SPOTS %	+ INDIVIDUALS ♀	+ INDIVIDUALS ♂	+ SPOTS
(1) *My/ru h th st p^p sr e^s*	2757	2564	42	24	—	1.2	3837	3495	—
(2) *My/Sb*	980		8	2	2	1.2	—	—	—
(3) *Sb Mw/ru h th st cu sr e^s ca*	1348	1526	46	38	—	2.8	—	—	—
(4) *Mw/Sb*	419		—	—	3	0.7	461		3*
(5) *Mw/ru h th st p^p cu sr e^s*	580	647	3	7	1	0.8	—	—	—
(6) *Mw/h st th sr e^s ro ca*	583	525	4	1	—	0.4	—	—	—
(7) *Mβ/ru h th st sr e^s ca*	1487	1240	6	1	—	0.3	1579	1345	—
(8) *M33j*/+(6 separate experiments)	1915	—	1	—	—	0.05	—	—	—
Total ♀ (excl. exp. (2) (4) (8))	**6755**	—	**110**	—	+(?)	**1.6**	**5416**	—	—
Total ♂ (excl. exp. (2) (4) (8))	—	**6502**	—	**73**	+(?)	**1.1**	—	**4840**	—
Grand total	—	**16571**	—	—	**189**	—	**1.1**	**10717**	—	**3**

* Spot on ♀: 1.
Spot on ♂: 1.
Spot on sex unknown: 1.
All these had $+^{Sb}$ setae. In one of them the setae were very small in addition.

mutants which are distinguishable in every single micro- or macrochaeta, like the sex-linked mutants *y* and *sn*^{3}, were available but the absence of the dominant Minute is recognizable in a single macrochaeta as is the absence of the dominant gene Stubble (*Sb*, III, 58.2). The recognition of the other genes used depends on the occurrence of multicellular areas in certain regions of the head and thorax.

In comparing the frequencies of autosomal spots (table 19) with those of sex-linked mosaic areas it is necessary to remember the different probabilities of discovering spots which become visible whenever they include a seta and spots which are phenotypically apparent under certain conditions only. It is, however, obvious that the frequency of third chromosome spots is considerably lower than that of comparable sex-linked spots in flies containing *Mn* or the Blond-Minute.

A similar difficulty arises in an evaluation of the frequency of autosomal spots in Minute flies as compared to that of controls which contain no Minute factor. As can be seen from tables 20–24, a large number of spots in Minute flies were recognizable only by the appearance of $+^M$ bristles In not-Minute controls such areas would not be different from the rest of the fly. It is certain that on the whole the presence of autosomal Minutes increases the incidence of autosomal spots as shown by the fact that no spots were found in 7332 not-Minute individuals, controls of experiment 1, nor in 2924 control flies in experiment 4. However, among the *Sb*/+ controls of experiment 4 three individuals occurred each of which possessed one not-Stubble bristle.

Of the three Minute factors studied, the locus of *My* is to the left of the fibre attachment point at 40.4, while that of *My* and *Mβ* is to the right of this point, at 80± and 85.4 respectively.

My. Two experiments involved *My*. In the first, *My* was present in one chromosome, while the following recessives were present in the homologous third chromosome: *ru*, 0.0 roughoid; *h*, 26.5 hairy; *th*, 42.2 thread; *st*, 44.0 scarlet; *p*^{p}, 48.0 peach; *cu*, 50.0 curled; *sr*, 62.0, stripe; *e*^{s}, 70.7, sooty. Phenotypically, such flies show only the Minute bristle condition. Table 20 indicates that 66 spots were found. In all cases (57 out of 66) where the spots enclosed one or more macrochaetae it was seen that these setae were normal in length, indicating the absence of *My*. Whenever the spots covered an area which in a fly homozygous for *h* would exhibit the extra-hair effect of this recessive gene extra hairs were present indicating the absence of the normal allele of *h* (45 cases). In 8 out of 9 cases, where the spot involved part of an eye, the recessive characters roughoid eye-texture and scarlet eye-color were visible, indicating the absence of the normal alleles of these genes. The arista of one antenna was involved 6 times and presented the phenotype of "thread," indicating the absence of

this gene's normal allele. In contrast to the appearance of the phenotypes of *ru*, *h*, *th*, *st*, and $+^{My}$ no spot presented the phenotypes peach eye-color and stripe and sooty body coloration. Peach would have been recognized in the 9 spots which covered part of an eye and sooty or stripe and sooty would have been recognizable in more than 20 spots. The phenotype curled wing did not occur either, but it is unknown if this is an autonomous character which would appear in mosaic spots.

While the foregoing description has taken account of each gene by itself, many spots in reality permitted the determination of the presence or absence of more than one character at the same time. This is to a certain degree obvious from an inspection of table 20. As a whole the findings show that spots produced in *My* flies exhibit all those recessive genes which are located in the $+^{M}$ carrying third chromosome to the left of a point between *st* and *p* and that the spots do not exhibit phenotypes of any genes located to the right of this point. The only exception is represented by one spot which covered part of the head and showed the phenotype *h*, *st*, and $+^{M}$ but not that of *ru*.

TABLE 20

Spots on My/ru h th st p^{p} sr e^{s} flies (cf. table 17).

$h+^{M}$		$ru\ h\ th\ st+^{M}$	$ru\ h\ st+^{M}$	$ru\ st$	$h\ st+^{M}$	$+^{M}$		h	th
1	>1	>2	>2	0	>2	1	>1	0	0
2	32	3	2	3	1	12	5	5	1

The second experiment which involved *My* concerned flies of the constitution *My/Sb*, with the phenotype Minute-y Stubble bristles (table 21).

TABLE 21

Spots on My/Sb flies (cf. table 17).

$+^{M}\ Sb$		$M+^{Sb}$		(?)$M\ Sb$
1	>1	1	>1	1
3	6	2	1	1

There were 13 aberrant areas. In 9 cases the phenotype *My* had disappeared, while that of Stubble was still present, in 3 cases the reverse was true, resulting in Minute, not-Stubble bristles. The 13th spot was formed by one bristle, which showed both Minute and Stubble characters although in abnormal fashion.

Mw. Before we discuss these mosaic spots induced by Minute-y, we shall describe the phenomena in flies possessing Minutes located in the right arm of the third chromosome. The largest experiment involved Minute-w, *Sb*, and the recessives *ru*, *h*, *th*, *st*, *cu*, *sr*, e^{s}, *ca* (*ca*, III, 100.7

claret; table 22: *Sb Mw/ru h th st cu sr e^s ca*). In 73 out of the total of 84 spots both *Sb* amd *Mw* had disappeared. Of these 73 spots 14 were large enough and were situated in suitable regions so that the presence or absence of e^s or e^s and *sr* was recognizable; indeed, these genes, originally present in heterozygous condition only, were phenotypically obvious in all 14 cases, as well as in 4 more areas (not included in the 73 areas) which did not involve macrochaetae, so that their nature in respect to *Sb* and *Mw* could not be tested. Two $+^{Sb}+^{M}$ spots involved part of the eye and were claret-colored. No single spot exhibited the phenotypes *ru*, *h*, *th*, or *st*, although *ru* and *st* would have been recognizable in 2 spots, *h* in at least 9 spots, and *th* in 1 spot. In total, $73+4=77$ spots can be interpreted as exhibiting when possible the phenotypes of all recessive genes located in the $+^{M}$ carrying third chromosome to the right of a point between *st* and

TABLE 22

Spots on Sb Mw/ru h th st cu sr e^s ca flies (cf. table 17).

$+^{Sb}+^{M}e^s$(or e^s sr)	e^s or e^s sr	$+^{Sb}+^{M}ca$	$+^{Sb}+^{M}$		$Sb+^{M}ca$	$Sb+^{M}$		$+^{sb}M$
	o	>1	1	>1	>1	1	>1	1
14	4	2	2	55	1	2	2	2

Sb, and they do not exhibit phenotypes of any genes located to the left of this spot. These findings are completely opposite to the findings regarding *My*.

There occurred 7 exceptional spots. They agreed with the rest by not exhibiting any genes located to the left of the region noted (1 case: $+^{ru}+^{h}$), but differed by showing either $+^{Sb}M$ or $Sb+^{M}$ bristles and in addition neither *sr* nor e^s (1 case).

A corroboration of these results is provided by 11 spots in flies of the constitution *Mw/ru h th st p^p cu sr e^s* and 5 spots in flies of the constitution *Mw/h th st sr e^s ru ca* (table 23). In 13 cases the recessive genes of the right half of the $+^{M}$ third chromosome were visible ($+^{M}$, e^s, or *sr*, e^s according to the region involved). In 6 out of these 13 cases the phenotypic absence of the left arm gene *h* was evident although its presence would have been recognizable. While these results agree with the general deduction which has just been made, the remaining 3 mosaic areas were exceptional again. They did not exhibit the phenotypes *sr* and e^s, in spite of location on suitable thorax regions. In two of these spots it could be ascertained that *h* did not appear. This finding agrees with that in all other spots.

A last experiment involving *Mw* concerned flies of the constitution *Sb/Mw* (419 individuals). Only 3 spots were found, two of which were phenotypically $Sb+^{M}$ (1 doubtful), the third being $+^{Sb}M$.

An experiment in which *M β* was present is reported in table 24. Of a

TABLE 23

Spots on Mw flies (cf. table 17).

CONSTITUTION	$+^M$ e^s (or e^s sr) o >2	e^s or e^s sr o	$+^M$ 1 >1	$+^M+^{e,sr}$ >1
Mw/ru h th st p^p cu sr e^s	3 —	4	1 1	2
Mw/h th st sr e^s ro ca	1 2	—	1 —	1

total of 7 spots 6 exhibited suitable recessive genes located in the right arm of the not-Minute third chromosome, while no spot with genes located in the left arm appeared (1 spot was definitely $+^h$). The seventh mosaic area was exceptional in having $+^M$ bristles but also $+^{sr}$, $+^e$ coloration (*h* did not appear either).

All findings regarding autosomal mosaic areas in flies carrying third chromosome Minutes can be summarized in the following statements:

(1) Spots exhibit only phenotypes of recessive genes, which are located in one arm of the not-Minute carrying chromosome. If the Minute factor is in the left arm of the third chromosome (*My*), only recessive genes in the left arm become visible; if the Minute factor is located in the right arm (*Mw*, Mβ), only recessive genes in the right arm exhibit their effect.

(2) In the majority of spots all genes located in the respective arm are involved.

(3) A smaller number of spots exhibits only the phenotypes of *some* genes located in one arm.

Originally these findings were explained by the elimination assumption. That arm of the Minute-bearing chromosome or part of it in which the Minute is located was thought to be broken off and lost from the cell. The following considerations make this assumption untenable now: (a) the one spot reported in table 20, which exhibited *h*, *st*, and $+^M$, but not *ru* can be explained by elimination only if one assumes the loss not of a whole arm or a whole end section of the chromosome but of a middle piece. Such a process would necessitate special assumptions with regard to elimination which seem rather artificial. It should be mentioned, however, that a slight possibility exists that the absence of the phenotype *ru* in the eye-spot may not really indicate the presence of the dominant $+^{ru}$ but may conceivably be an extreme phenotypic variation of the somewhat variable expression

TABLE 24

Spots in Mβ/ru h th st cu e^s ca flies (cf. table 19).

$+^M$ e^s (or e^s sr)	e^s or e^s sr	$+^M$	$+^M+^{e,sr}$ ($+^h$)
2	3	1	1

of *ru*. (b) As will be shown below, the occurrence of autosomal spots can be interpreted by the theory of somatic crossing over in autosomes. As the theory of somatic crossing over has been proven to be superior in the case of sex-linked mosaics, it is a priori probable that it holds also for autosomes. A direct test which would be provided by a demonstration of autosomal twin spots cannot be given, since autosomal spots occur hardly at all in not-Minute flies, while in Minute flies one of the segregation products after somatic crossing over becomes homozygous for the Minute factor and is not viable. Thus only a single, not a twin, mosaic area can appear.

Granting the validity of the theory of autosomal somatic crossing over and segregation, a detailed analysis of the data yields an unexpected result. We consider first those cases which form the majority and which demonstrate that the region of somatic crossing over is restricted to the neighborhood of the spindle fibre point. This point in the third chromosome is located in the middle, between scarlet (44.0) and pink (48.0, peach is an allele of pink). It was just this interval in experiment 1 (table 20) which divided the genes whose phenotypes appear in mosaic areas from those which did not appear; in experiment 3 (table 22) where the critical region could not be narrowed down as completely, but could only be said to lie within the interval *st* (44.0) to *Sb* (58.2) the spindle fibre point is again included. Restricting our analysis to single crossovers between two of four strands only, there are four possible crossing over and segregation processes which lead to the appearance of somatic areas:

(1) *Crossing over to the left of the fibre point.*

(x) If *A B* S′ *C D/a b* S² *c d* is the original constitution of the autosomes, S′ and S² representing homologous fibre points, segregation of the four chromatids after crossing over with normal equational separation of the daughter fibres **(x)** yields: *A B* S′ *C D/A B* S² *c d* and *a b* S′ *C D/a b* S² *c d*. The latter constitution would be visible as a mosaic area exhibiting *a* and *b*.

(y) Non-disjunctional separation of the daughter fibres **(y)** yields *A B* S′ *C D/a b* S′ *C D* and *A B* S² *c d/a b* S² *c d*, the latter exhibiting *c* and *d*.

(2) *Crossing over to the right of the fibre point.*

(x) segregation results in *A B* S′ *C D/a b* S² *C D* and *A B* S′ *c d/a b* S² *c d*, yielding a spot *c d*.

(y) segregation results in *A B* S′ *C D/A B* S′ *c d* and *a b* S² *C D/a b* S² *c d*, yielding a spot *a b*.

An *a b* spot therefore occurs either as a consequence of crossing over to the left of the fibre point and equational fibre segregation (process **1x**) or as a consequence of crossing over to the right and non-disjunctional separation (process **2y**). Correspondingly a spot *c d* occurs either in conse-

quence of crossing over to the right of the fibre point and equational fibre segregation (process **2x**) or after crossing over to the left of the fibre point and non-disjunctional separation (process **1y**). It is believed that only one of each alternative is actually realized and that this involves normal equational separation of daughter fibre points. Reasons for this assumption are (1) the analogy with somatic segregation of X chromosomes in which equational separation has been demonstrated to be the rule and (2) the demonstration to be given in the next paragraph that equational separation of fibre points is the only method followed after somatic crossing over distal to the fibre region in the third chromosome.

An analysis of the smaller group of spots which exhibit only the phenotypes of some genes located in one third chromosome arm leads to an interpretation which considers these areas as caused by consequence of crossing over in regions other than that including the spindle fibre point. Single crossovers are sufficient to yield the different spots reported in columns 6, 7 and 8 of table 22, and the last columns of tables 23 and 25. All these spots occurred in experiments which involved Minute factors in the right arm of the chromosome. The locus of crossing over can be placed to the right of *e* (70.7) and to the left of *Mw* (80.0) or *Mβ* (85.4) respectively; this is judged from the fact that all recessive loci to the left of the *e-M* region failed to appear in mosaic areas, while those to the right did appear. Non-disjunctional segregation would have yielded areas which exhibit all recessive genes located to the left of the crossover point, thus including loci from both arms of the chromosome. But no such areas did occur. It can be demonstrated that these cases of somatic crossing over can have been followed only by equational fibre point segregation which would lead to the observed types of spots.

There remains a discussion of the few spots not yet dealt with. If the one *h st*$+^{M}$ spot of table 20 can be regarded as genotypically $+^{ru}$ as indicated by its phenotype, a case of double crossing over would be represented, the crossover points being located in the regions *ru-h* and *st-p*. A double crossover between the fibre point and *Sb* and between *Sb* and *Mw* would explain the $+^{Sb}Mw$ spots of table 22 and the $+^{Sb}Mw$ spot mentioned in the last paragraph of the section on *Mw*. The *My*$+^{Sb}$ areas recorded in table 21 can be regarded as crossover products to the right of the fibre point. Such spontaneous crossovers are believed also to be the explanation of the occasional occurrence of $+^{Sb}$ spots in not-Minute flies (table 19 (4), control). An alternative explanation assuming crossing over to the left of the fibre point would demand non-disjunctional fibre segregation. For the exceptional spot recorded in the last column of table 21 and the $+^{Sb}$ *M*-like spot among the controls of experiment 4, table

19, no discussion of hypothetical interpretations seems warranted.

The unexpected result brought out in the analysis of areas involving third chromosome genes consists in the definite correlation between the location of the Minute factor in the left or right arm of the chromosome and the corresponding location of the crossover point. No well-founded theory of this relation can be given.

In addition, it seems noteworthy that the distribution of crossovers along the chromosome is greatly changed as compared to germinal crossing over. The great majority of somatic crossovers seems to fall within the interval *st-p* which comprises less than four per cent of all germinal crossovers. As this interval encloses the fibre attachment point it represents the region adjacent to this point. The concentration of somatic crossovers in these regions parallels our former findings concerning the massing of somatic crossovers in the region adjacent to the fibre point of the X chromosome.

The occurrence of autosomal crossing over in females and males

Autosomal mosaic areas appeared both on females and on males. Somatic crossing over in autosomes is thus shown to occur in both sexes. However, it is seen from table 19 that a probably significant lower frequency was found in the males.

It may be added that an experiment was made to test the question whether an autosomal Minute would induce crossing over in its chromosome in the germ cells of males. Males of the constitution *Mw/h st cu, sr, e^s ca* were backcrossed to homozygous *h st cu sr e^s ca* females. None of the 4151 F_1 individuals were crossovers (class *Mw* = 2092, class *h st cu sr e^s ca* = 2059). There is then either no effect of *Mw* or the effect is too small to become apparent in this sample of more than 4000 flies. The experiments on induction of crossing over in the male of *Drosophila melanogaster* by FRIESEN (1934), PATTERSON and SUCHE (1934) and A. F. SHULL and WHITTINGHILL (1934) by means of X-rays or heat yielded positive results with much smaller numbers.

MOSAIC AREAS IN FLIES HETEROZYGOUS FOR X CHROMOSOME INVERSIONS

Whenever an X chromosome was present in our experiments which carried the bobbed-deficiency, described by SIVERTZEV-DOBZHANSKY and DOBZHANSKY (1933), unexpected peculiarities relating to mosaic areas were found. Attempts at interpretation met difficulties until it became known that the *bb^{Df}* chromosome, besides lacking a section of a normal X chromosome, carries a long inversion with the end points at 9± and

TABLE 25a

Spots in experiments involving primarily y, sn^3, and bb^{Df}. No Y chromosome present.

EXP.	CONSTITUTION*	IND.	SPOTS	NO SPOTS	sn^3 1	sn^3 2	sn^3 >2	y 1	y 2	y >2	$y\ sn^3$ 1	$y\ sn^3$ 2	$y\ sn^3$ >2	y-sn^3 2	y-sn^3 >2	y-$sn^3\ y$ 2	+♂ COL.
1a	$y\ bb^{Df}/sn^3$	884	12†		6	—	2	4	—	—							
		340	28		8	2	—	12	3	3							
		683	153		34	8	11	62	16	10				4	7		1
		122	18		2	—	1	7	3	2				2	—		1
					50	10	14	85	22	15				6	7		
Totals Exp. 1		**2029**	**211**	**10**	**74**			**122**			—			**13**			**2**
2	$y\ sn^3\ bb^{Df}/+$				2	—	—	6	3	3	19	1	1			1	1
		838	37	4	2			12			21			—		1	1
3a	$sn^3\ bb^{Df}/y$	821	44·		23‡	1(?)	—	14	4	2	—			—		—	—
					24			20									
		22	20		3	1	—	8	4	—	—			3	1	—	—
					4			12						4			
		29	6		2	—	1	—	1	1	—			—	1	—	—
					3			2						1			
				8§	28	2	1	22	9	3				3	2		
Totals Exp. 3		**872**	**70**	**(6)§**	**31**			**34**						**5**			
4	$bb^{Df}/y\ sn^3$	740	15	2	1	—	(1?)	3	2	1	1	1	4	—	—	—	1
					2			6			6						

* Besides the genes indicated, other recessive sex-linked factors were present in heterozygous condition in some experiments. An autosomal Minute was present in some of the flies of exps. 1a, b, c. In exp. 3c and partly in exp. 4 the constitution in respect to the *bb* locus was bb^{Df}/bb.

† Inspected for head and thorax spots only.

‡ Two doubtful.

§ The value of 6% is obtained if one excludes exp. 3b which represents individuals of one single culture which, raised at the same time with 29 cultures recorded as exp. 3a, gave an exceptionally high number of *y* spots.

64± (BEADLE and STURTEVANT 1935). On the basis of this fact an explanation of the observed spots could be given. Furthermore, in the course of working with flies containing bb^{Df} it was found that bb^{Df} stocks frequently contained supernumerary Y chromosomes as had been described for an independent bb^{Df} stock by GERSHENSON (1933). This led to the discovery of a striking influence of the Y chromosome on somatic crossing over. Accordingly the results for each experiment involving a known constitution of the X chromosomes will be given separately for those cases in which no Y chromosome was present in the females and those in which a Y chromsome was present. Special crosses had to be undertaken in order to assure the absence or the presence of an extra Y chromosome and in many cases progeny tests were carried out as independent tests.

Experiments involving y, sn^3, and bb^{Df}; no Y chromosome present

As in foregoing analyses, all four possible combinations of the three allelic pairs were secured so that any interpretation has to stand a fourfold test. The results are given in tables 25a and b. If we compare them with the similar experiments involving *y* and sn^3 recorded in tables 5 and 8 we note (a) the relatively low number of spots with 2 or more setae, (b) the relatively low number of twin spots in experiments 1 and 3, (c) the relatively high number of spots other than *y* sn^3 in experiments 2 and 4. A further very striking fact is not indicated in table 25: although the setae in non-mosaic areas were of normal size, the majority of *y* sn^3 setae in experiment 2 and of the sn^3 setae in experiment 3 were of very small size varying from fine, thin structures to larger but still distinctly subnormal ones. A similar picture was presented by many *y* setae in experiment 1. It was different with at least part of the *y* setae in experiments 2 and 3,-as well as with most of the different setae in experiment 4; they were of normal size.

The explanation of these phenomena is based on MCCLINTOCK'S (1933) cytological findings with reference to crossing over involving chromosome inversions. Her results have been confirmed by later workers (MÜNTZING 1934; SMITH 1935; MATHER 1935; HÅKANSSON 1936) and have been applied to a genetic analysis by BEADLE and STURTEVANT (1935). We shall restrict our discussion to the consideration of single crossovers and equational fibre point segregation, processes which can account for the great majority of spots. Crossing over can occur in two different sections of the X chromosome, outside the inverted region and inside. The first section in a chromosome carrying the bb^{Df} inversion is formed by the left end of the X chromosome, from *y* to about the locus 9, the second section by the rest of the chromosome. Whenever somatic crossing over occurs between the left ends of the chromosomes we obtain the same results as discussed

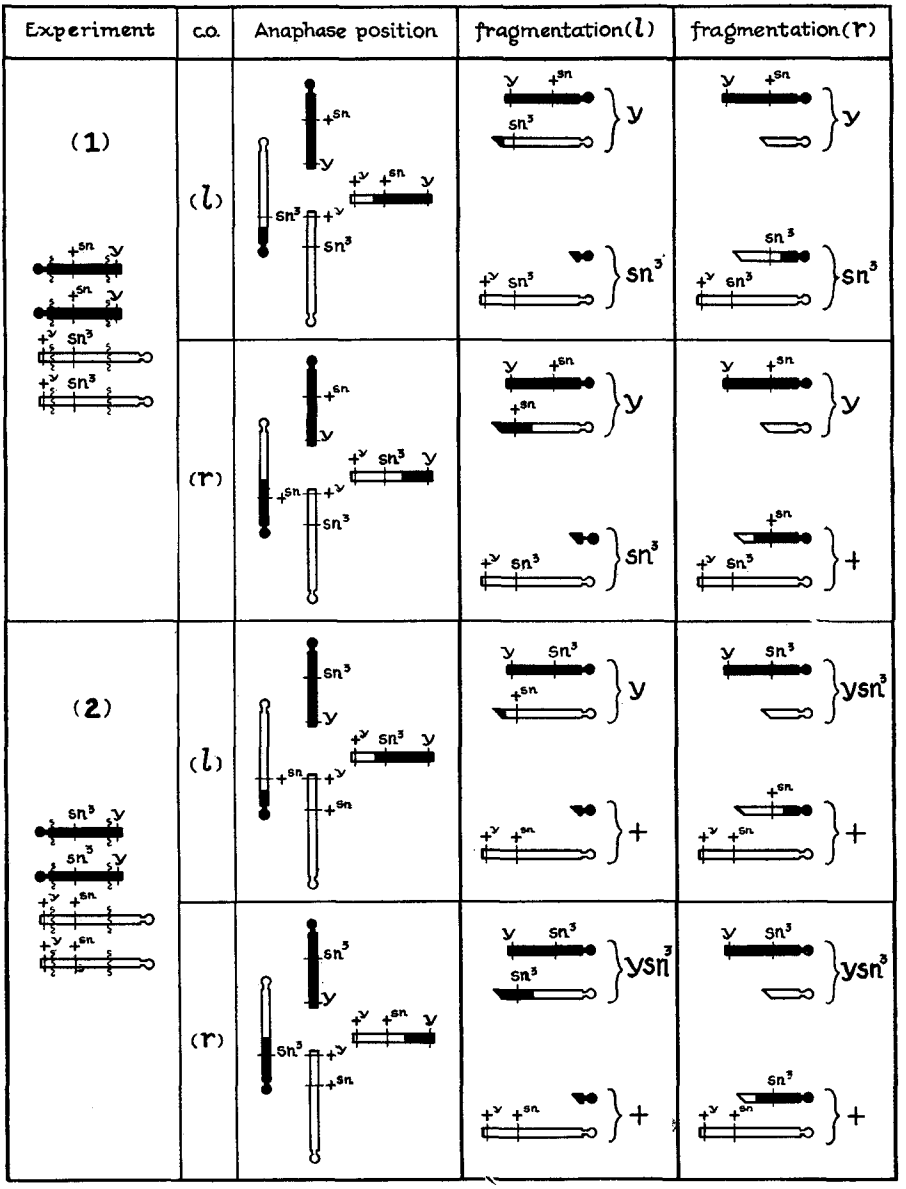


FIGURE 10 a, b. Four experiments involving *y*, *sn*^{3} and *bb*^{Df}. (See tables 25–27, experiments 1, 2, 3, 4.) The results of x-segregation after crossing over to the left (l) and to the right (r) of *sn*^{3} and of fragmentation of the chromatid with two fibre points to the left (l) and to the right (r) of *sn*^{3}. The lines } indicate the end points of the inverted region.

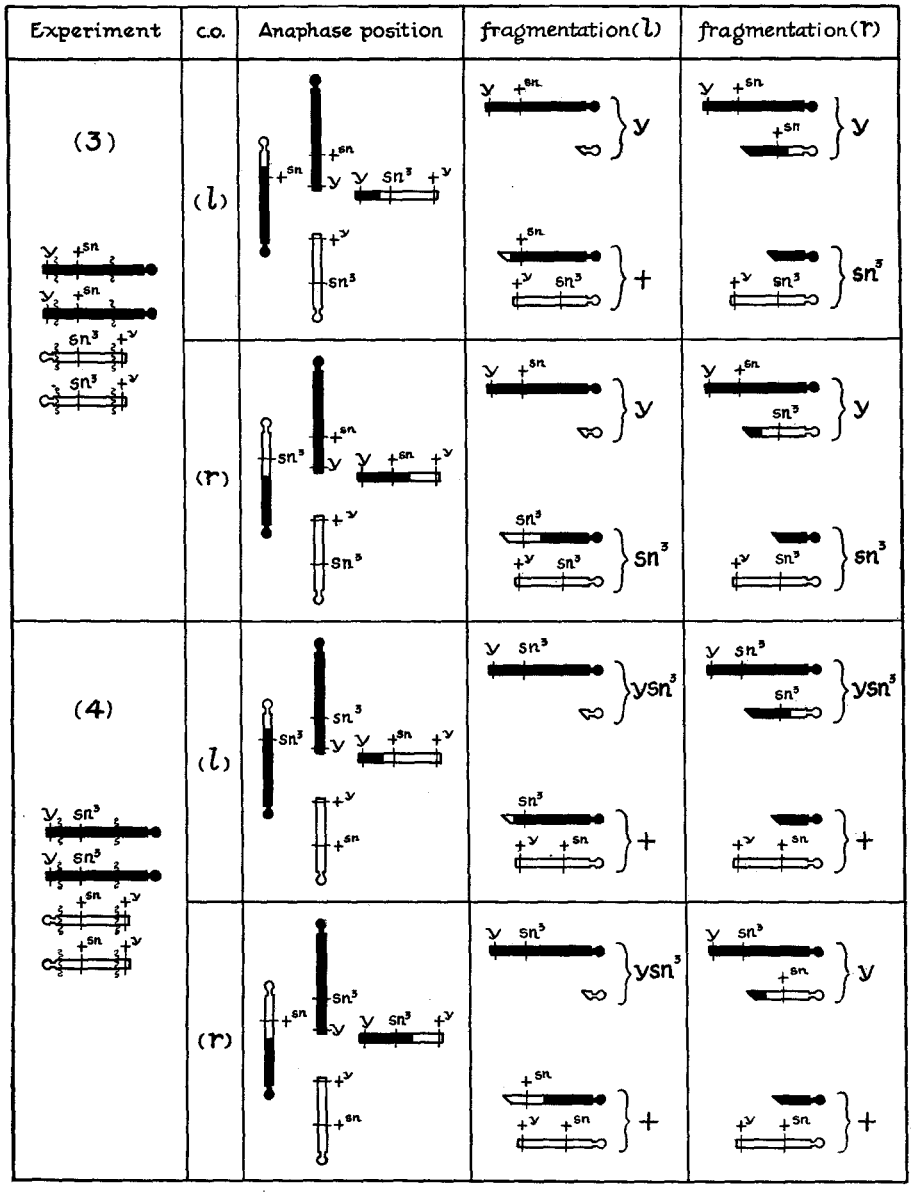


FIGURE 10b (see opposite)

in an earlier chapter, namely, in all four experiments, the appearance of single *y* spots. The spots contain two X chromosomes and the phenotype of these *y* setae is expected to be normal. Part of the *y* spots with setae of normal length are believed to have originated in this way.

A very different situation is produced as a consequence of somatic crossing over within the inverted region (fig. 10). If crossing over occurs at a four strand stage, two normal non-crossover strands will be formed, while the two other strands will yield one strand containing no spindle fibre point and one containing one fibre point on each end (fig. 10). If segregation is accomplished by equational disjunction of sister fibre points, two possible distributions result.

TABLE 25b

Sexual coloration of critical spots from table 25a.

EXP.	*sn*^{3}		*y*		*y sn*^{3}	+	TOTAL	
	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.
1b	1	—	—	—	—	—	15	3
c	5	2	9	—	—	1		
2	—	—	1	—	—	1	1	1
4	—	—	1	—	1	—	1	1
Totals							17	5

(1) Segregation **(z).** Both non-crossover strands go to one pole and the crossover products to the other pole. The chromatid without any attachment is expected to be eliminated. In this case one cell obtains a constitution not different from that of any non-crossover cell, while the other cell will receive the chromatid with two attachment points. This cell will lack completely the left end of the X chromosome and presumably would not give rise to a viable product. Thus, segregation **(z)** would not yield visible mosaic areas.

(2) Segregation **(x).** In this case the two non-crossover chromatids segregate to opposite poles, while the chromatid with two attachment points becomes oriented parallel to the spindle axis in such a way that sister fibre points are directed toward opposite poles. The chromatid without any fibre point is again regarded as being left in the middle of the mitotic figure and thus eliminated. There are two ways by which such a process might result in two daughter nuclei.

First, it is possible that the double-fibre-point chromatid will not be included in the nuclei and so be eliminated. In this case cel s with the following constitutions will be formed:

In experiment (1) twin cells with *y* bb^{Df} and sn^3
" " (2) " " " *y* $sn^3 bb^{Df}$ and +
" " (3) " " " sn^3 bb^{Df} and *y*
" " (4) " " " bb^{Df} and *y* sn^3

In all cases only one X chromosome will be present in each nucleus. The cells with bb^{Df} are not expected to survive. Thus single mosaic areas would be formed of the constitutions sn^3, +, *y*, or *y* sn^3. Actually a process of elimination cannot account for more than a very small number of spots, at best. For it does not account at all (a′) for the abnormally small size of setae in the majority of spots, (b′) for the $+^{sn}$ spots in experiment 1 (125 out of 209), (c′) for none except 1 spot (out of 37) in experiment 2, (d′) for the $+^{y}$ spots in experiment 3 (36 out of 70), (e′) for the not-*y* sn^3 spots in experiment 4 (9 out of 15), and (f) for the fact (table 25b) that out of a total of 22 spots in which the sex could be determined only 5 showed male coloration whereas the elimination process should lead to male spots only. The same arguments dispose also of the possibility that the two-fibre-point chromatid as a whole often becomes included in one of the daughter nuclei. In such cases the sister nucleus would have a 1X constitution as outlined above.

Let us therefore consider a second possibility. Again we regard the case that the two non-crossover chromatids go to opposite poles and that the two-fibre-point chromatid is arranged so that sister fibre points of the chromosome group segregate equationally. The two-fibre chromatid will then be subjected to conflicting forces, one end with its fibre attachment "pulling" in the opposite direction from the other. On the basis of the cytological observations of the authors named above, who studied two-fibre chromatids in meiosis we assume that the two-fibre chromatid breaks at some point under the stress and that the two fragments become included, each in one daughter nucleus. Such a hypothesis leaves room for two pairs of alternative possibilities resulting in four types of areas to be expected (fig. 10). The two-fibre chromatid will either contain sn^3 or $+^{sn}$ according to the region in which crossing over occurs in the inverted section, namely to the left of sn^3 (as measured in the non-inverted chromosome) or to the right of it. Also, the fragmentation break can fall on either side of the locus of sn^3. The consequences of these four possible occurrences are given in figure 10. Sister cells originate in each case which possess one complete X chromosome (disregarding the deficiency for *bb*) and one X fragment of varying length.

No prediction can be made as to the relative frequencies of the four processes. Crossing over to the left of sn^3 can occur in the section from locus 9–21 only, while crossing over to the right can occur between the loci 21 and 64. This fact would seem strongly in favor of crossing over to

the right of the sn^3 locus. But as both the region to the left and to the right of sn^3 extends up to a fibre point in one of the two chromosomes the incidence of crossing over on the two sides of sn^3 might tend to become more equal. The presence of the bb^{Df} introduces another factor of unknown influence. With regard to the fragmentation break it may seem that it should preferably fall to the "right" of the sn^3 locus (measured in comparison to the non-inverted chromosome), the more so as the "right" section is not only so much longer than the "left" one but includes in addition to the active region the long inert part which on account of the *bb*-deficiency is absent on the other side of sn^3. However, no a priori reasoning should be applied to this situation because the effect of the inert material on the mechanical properties of the X chromosome is an open question since SCHULTZ'S (1936) and OFFERMANN'S (1936) discovery of visible cytological differences in X chromosomes lying in salivary gland nuclei with or without extra inert material.

If we want to compare our actual findings with the expectations from the proposed scheme we must call attention to one more point. All nuclei originating from the different processes possess unbalanced X-chromosomal constitutions. Each contains only once the normal left end of the X chromosome, from *y* to the beginning of the inversion, but each contains certain other regions in duplicated condition, once in the non-crossover chromatid and once in the chromosome fragment. The degree of unbalance is variable according to the breakage point of the fragmenting chromatid. *Zygotes* with such unbalanced constitution will, in most cases, not be able to give rise to viable individuals, but it is permissible to assume a certain degree of viability of *hypodermal areas*. This leads us to expect mosaic areas originating from these processes to be small in size and covered with setae of varying degrees of abnormal growth. In many cases it is to be anticipated that the viability of two sister cells is different so that only one will be able to give rise to a mosaic area. Three striking phenomena regarding table 25a are seen at once to be in agreement with such expectations, namely the facts (a) "relatively low number of spots with 2 or more setae in experiment 2 and 4," the fact (b) "relatively low number of twin spots in experiments 1 and 3," and the finding that the majority of setae were abnormally small. Furthermore the genetic type of spots found in each of the four experiments agrees with the expectations (fig. 10). Only very few spots occur which cannot be accounted for on the assumption of single crossing over within the inversion and fragmentation of the two-fibre-point chromatid; but these spots are to be expected from crossing over outside of the inversion and from multiple crossovers. It cannot be stated with certainty whether the four theoretical possibilities

concerning the region of crossing over and of fragmentation are all realized. Experiment 2 indicates that the great majority of spots, the *y sn*^{3} spots, originated as a result of one or all of the processes "crossing over to the left of *sn*^{3}, fragmentation break to the right of *sn*^{3}" **(l,r),** "crossing over right, fragmentation left" **(r,l),** and "crossing over right, fragmentation right" **(r,r).** The *y* spots with small setae, which occurred in the same experiment, point to the occurrence of the fourth process "crossing over left, fragmentation left" **(l,l).** In experiment 4 the presence of *y* spots seems to point to the occurrence of process **r,r.** If then we take the representation of processes **l,l** and **r,r** for granted, we have established the position of crossover and of fragmentation points to both sides of *sn*^{3} in two combinations. There seems no objection to the assumption that the two other combinations, **l,r** and **r,l** are also realized. An attempt to evaluate the frequency of the four types on the basis of the actual data fails due to the variability of the results.

The total frequency of spots is higher in experiments 1 and 3, namely 10 per cent and 8 (or 6) per cent, respectively, as compared to experiments 2 and 4 where it is 4 and 2 per cent. This agrees in a general way with the fact (fig. 10) that in experiments 1 and 3, 7 out of the 8 segregational constitutions can potentially give rise to visible mosaic areas, while in experiments 1 and 4 only 4 out of the 8 constitutions can lead to visible spots.

Table 25b showed that 17 out of 22 spots in which the sex could be recognized were female and the remaining 5 were male. In these experiments both types of spots are to be expected, for a very short fragment in addition to the one X chromatid should result in a male spot and the presence of a longer fragment in a female spot.

Summary: The hypothesis of somatic crossing over in a heterozygous inversion accounts for the observed mosaic spots if it is assumed that the resulting chromatid without any fibre point is eliminated and the chromatid with two fibre points is fragmented so that each of the two fragments with its fibre point is included in a daughter nucleus.

Experiments involving y, sn^3*, and bb*^{Df}*; a Y chromosome present*

Females with essentially the same constitution in regard to X-chromosomal genes as those reported in the preceding chapter, but possessing a Y chromosome, were obtained and exhibited the spots summarized in tables 26a, b. A comparison of the two groups, without and with Y chromosome (table 27) yields three results:

(a) The presence of a Y chromosome increases the frequency of spotting. The percentages for the four pairs of experiments are:

TABLE 26a

Spots in experiments involving primarily y, sn^3 and bb^{Df}. A Y chromosome was present.

EXP.	CONST.*	IND.	SPOTS	% SPOTS	sn^3 1	sn^3 2	sn^3 >2	y 1	y 2	y >2	y-sn^3 1	y-sn^3 2	y-sn^3 >2	y-sn^3 2	y-sn^3 >2	y-ysn^3 2	y-ysn^3 >2
1a	$y\ bb^{Df}/sn^3$ Y	161	62	—	3	1	3	36	12	7							
b		19	9	—	—	—	—	3	1	5							
					3	1	3	39	13	12							
Total		**180**	**71**	**39**	**7**			**64**									
2	$y\ sn^3\ bb^{Df}/+$Y	507	145	29	5(1?)	1	—	11(1?)	3	—	53	36	34	—		1	1
Total					**6**			**14**			**123**					**2**	
3	$sn^3\ bb^{Df}/y$ Y	458	57	12	34(3?)	1	2	10	2	5	—			2	1	—	
Total					**37**			**17**						**3**			
4	$bb^{Df}/y\ sn^3$ Y	863	50	6	10	3	3	12	5	10	4	—	3	—		—	
Total					**16**			**27**			**7**						

* All flies in exp. 1a contained an autosomal Minute. In exp. 1b and 4 the constitution in regard to the *bb*-locus was bb^{Df}/bb.

Experiment	*Without Y*	*With Y*
1	10	39
2	4	29
3	8 (or 6)	12
4	2	6

(b) The relative frequencies of different types of spots are changed by the presence of a Y chromosome.

(c) No phenotypes of spots in flies carrying a Y chromosome occur which did not also occur in flies without a Y chromosome.

A closer examination shows that the increase of total frequency of spots under the influence of a Y chromosome is mainly due to the rise of one particular class in each experiment. The classes with the heightened frequency are in experiment 1: *y*; in 2: *y sn*^{3}; in 3: *sn*^{3}. In experiment 4 two classes, *sn*^{3} and *y*, have been increased.

TABLE 26b

Sexual coloration of critical spots from table 26a.

EXP.	*sn*^{3} ♂ COL.	*y* NO ♂ COL.	*y sn*^{3} NO ♂ COL.	*y sn*^{3} ♂ COL.	TOTAL NO ♂ COL.	TOTAL ♂ COL.
1b	—	1*	—	—	1	0
2	—	—	27(1?)	1	27	1
3	1	1	—	—	1	1
4	—	6	1	1	7	1
Total					**36**	**3**

* This spot comprised a large part of the abdomen.

The data can be accounted for by the hypothesis that the presence of a Y chromosome leads to a higher frequency of the crossing over and fragmentation process **r,r** (fig. 10). This is obvious in experiments 1 and 2 where only *y* and *y sn*^{3} spots will become more numerous. In experiment 3 where the process **r,r** results in *y* and *sn*^{3} sister cells the further assumption is necessary that the viability of the *y* segregate is so much lower than that of the *sn*^{3} segregate as to result mainly in *sn*^{3} single spots. This assumption is compatible with the data in experiments 1 and 2. In experiment 4 the hypothesis leads to the expectation of an increase in + segregates which are not recognizable. According to this the higher frequency of *sn*^{3} and *y* spots in 4 has to be accounted for in a different way. No explanation for this increase will be advanced as the rather low numbers would make any attempt unsafe. But it may be pointed out that apart from the very striking increase of the classes *y* and *sn*^{3} in experiments 1 and 2, a slight increase is visible also in the percentage of some other spots.

The spots occurring in experiments with the Y chromosome are larger than in those without a Y chromosome (tables 25a and 26a). This is reflected especially in the fact that 70 out of 123 *y sn*^{3} spots in experiment 1, table 26a, covered 2 or more setae. The significance of this result is doubtful.

The interpretation of the influence of a Y chromosome on somatic crossing over by means of a preferential increase in process **r,r** leaves still open the question as to the underlying mechanism. The occurrence of segregational products in connection with the process **r,r** is dependent on three conditions: (1) crossing over to the right of *sn*^{3} and (2) fragmentation to the "right" of *sn*^{3}. Both conditions must be involved in causing the increase

TABLE 27

Summary of spots from experiments involving primarily y, sn^3*, and bb*^{Df}*, without or with the presence of a Y chromosome. Details in tables 25, 26.*

EXP.	CONST.	IND.	SPOTS	% SPOTS	*sn*^{3}	*y*	*y sn*^{3}	*y*-*sn*^{3}	*y*-*y sn*^{3}	♂ COL. +
1	*y bb*^{Df}/*sn*^{3}	2029	211	10	74	122	—	13	—	2
	y bb^{Df}/*sn*^{3} Y	180	71	39	7	64	—	—	—	—
2	*y sn*^{3} *bb*^{Df}/+	838	37	4	2	12	21	—	1	1
	y sn^3 *bb*^{Df}/+Y	507	145	29	6	14	123	—	2	—
3	*sn*^{3} *bb*^{Df}/*y*	872	70	8(6)	31	34	—	5	—	—
	sn^3 *bb*^{Df}/*y* Y	458	57	12	37	17	—	3	—	—
4	*bb*^{Df}/*y sn*^{3}	740	15	2	2	6	6	—	—	1
	bb^{Df}/*y sn*^{3} Y	863	50	6	16	27	7	—	—	—

of the process **r,r**: higher frequency of condition 1 can be effective only if accompanied by higher frequency of 2. A rise of 2 by itself would not be compatible with the observed total increase of spots as it would mean only an increase in number of **r,r** processes at the cost of a decline of **r,l.** The result of these deductions is the hypothesis that the influence of a Y chromosome on the frequency of certain spots is accomplished by an increase in the frequency of somatic crossing over to the right of *sn*^{3} (in the normal X chromosome) combined with an increase in fragmentation frequency in that part of the two-fibre-point chromatid which lies between *sn*^{3} and the normal (not-*bb*^{Df}) end of the chromatid. A third element, a possible rise in the frequency of **x**-segregation at the expense of **z**-segregation can at best be only an additional factor. Further data regarding the influence of a Y chromosome will be discussed in following chapters.

An exceptional case of segregation in experiments involving y, sn³, bb^Df and an extra Y chromosome

All fourteen experiments recorded in tables 25a and 26a gave results which agreed among each other. A fifteenth experiment, however, had a fundamentally different outcome. The flies in this case were of the same constitution as those in "experiment 1 with Y," namely *y bb^Df/sn³*; Y. They also contained the autosomal Minute 33j in heterozygous constitution, but so did many of the females of experiment 1a, table 26a, first line. Table 28 records the results which were obtained from a set of 7 cultures

TABLE 28

Spots from an exceptional experiment involving y, sn³, bb^Df and a Y chromosome.

IND.	SPOTS	%	*sn³* 2	*y* 1	*y* 2	*y* >2	*y-sn³* >2	*y-sn²* >2	SEX OF SPOTS (ALL *y*)* NO ♂ COL.	♂ COL.
146	277	191	1	98	59	115†	3	1(?)	—	—
—	—	—	1		272		3	1(?)	4	33‡

* In addition: 1 spot of the *y-sn³* twin spots showed ♀ coloration in the *sn³* part.

† 1 spot possibly belongs to the *y-sn³* twin spot class.

‡ 1 spot which covered the 6th right tergite had ♀ coloration laterally but not toward the median.

with two or three female and three male parents each. They were started several months apart from the experiments of table 26a.

The results deviate in three ways from those discussed in the last chapter: (a) 272 out of 277 spots belong to only one class; (b) the setae in spots were of normal size; (c) 33 out of 37 *y* spots were of male constitution.

It is unknown in which respect the flies in this experiment deviated fundamentally from those discussed above. That they contained a *y bb^Df* and a *sn³* X chromosome as well as a Y chromosome is certain, both from the parental constitutions and from progeny tests of 10 individuals. Although it has not yet been possible to duplicate the results they have been presented here as the numbers involved leave no doubt as to their significance. Besides, it is possible to account for them by the following assumptions: if, for reasons unknown, pairing between the genetically active regions of the two X chromosomes was inhibited and if pairing and somatic crossing over between the homologous regions of the X and Y occurred, then only the *sn³* bearing X and the Y would be involved. This is because the *bb^Df* chromosome lacks the region which finds its homologue in the Y chromosome. Equational segregation of the three pairs of fibre points will result in a *y bb^Df* chromatid going to each pole and in addition, in case of **x**-segregation of the crossover tetrad, in two *sn³* X chromatids going to one pole

and two Y chromatids going to the other pole (fig. 11). The result will be 3X and 1X sister cells. The 3X cells and their descendants do not form visibly mosaic areas on account of their normal phenotype, while the 1X cells form *y* areas. The size of setae in these areas is normal, as the deficiency for *bb* in the X chromosome is "covered" by the Y chromosomes and no extra X chromosome fragment is present. The sex of *y* spots, if recognizable, will be male. It is obvious that the hypothesis fits most of the facts but it should be remembered that no independent tests of it are available at present. Spots other than *y* with male constitution may

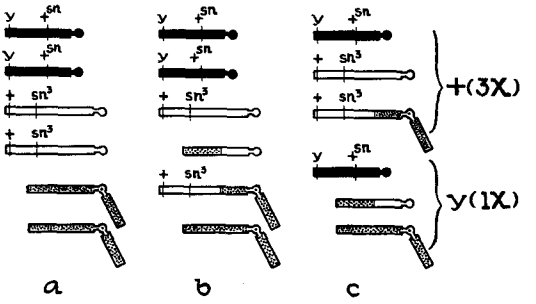


FIGURE 11. An hypothesis to account for the exceptional results in an experiment with *y* bb^{Df}/sn^3; Y flies. a. The six chromatids (2 bb^{Df}; 2 normal X; 2 Y). b. Four non-crossover chromatids and two chromatids resulting from crossing over between one normal X chromatid and one Y chromatid. c. The result of x-segregation of the six chromatids.

be thought to have originated in consequence of occasional crossing over between the X chromosomes as discussed in the foregoing chapter.

Experiments involving y, sn^3, bb^{Df} and Theta; no Y chromosome present

The hypothesis of the formation of a chromatid with two fibre points through crossing over within the inverted section, and the subsequent fragmentation of this chromatid, provided an explanation of the observed mosaic areas. It may, however, be asked if the differences in types of spots which occurred in the experiments with bb^{Df} as compared with those in which no inversion was involved were really great enough to warrant the elaborate scheme presented. Although the evidence presented seems to be in agreement with the hypothesis, further data will be adduced in the following sections which strengthen it greatly.

An analysis of the data given in table 29a, experiments 1 and 2 might profitably start with a comparison of the types and relative frequencies of spots in the experiments tabulated in table 13. There as here *y*, sn^3, and Theta were involved and the only difference consists in the presence or absence of the chromosome aberration connected with bb^{Df}. The results

TABLE 29a

Spots in experiments involving primarily y, sn^3, bb^{Df}, and θ, without or with a Y chromosome.

EXP.	CONSTITUTION	IND.	SPOTS	% SPOTS	sn^3 1	sn^3 2	sn^3 >2	sn^3 total	y 1	y 2	y >2	y total	$y\ sn^3$ 1	$y\ sn^3$ 2	$y\ sn^3$ >2	$y\ sn^3$ total	y-sn^3 2	y-sn^3 >2	y-sn^3 total	+♂ COL.	OTHER SPOTS‡
1	$\frac{y\ +\ \theta}{y\ sn^3\ bb^{Df}}$ no Y	392	702	180	98	81	203	382	102	30	22	154	11	5	9	25	18	95	113	13	15
1′	$\frac{y\ +\ \theta}{y\ sn^3\ bb^{Df}}$ Y	19	72	379	11	10	21	42	7	4	1	12	1	1	2	4	—	9	9	—	5
2	$\frac{y\ +\ bb^{Df}}{y\ sn^3\ \theta}$ no Y	262	53	20	2	—	—	2	33	8	6	47	—	—	—		—	—		4	—
2′	$\frac{y\ +\ bb^{Df}}{y\ sn^3\ \theta}$ Y	203	167	82	1	2	4	7	60	32	48	140	—	—	—		1	1	2	18	—

‡ Exp. 1. sn^3-$y\ sn^3$ twin spots: 8. y-$y\ sn^3$ twin spots: 4. y-sn^3-$y\ sn^3$ triple spots: 3.
Exp. 1′. sn^3-$y\ sn^3$ twin spots: 2. y-$y\ sn^3$ twin spots: 2. y-sn^3-$y\ sn^3$ triple spots: 3.
Exp. 2. y-+♂ col. twin spots: 2.
Exp. 2′. y-+♂ col. twin spots: 4. y-sn^3-+♂ col. triple spot: 1.

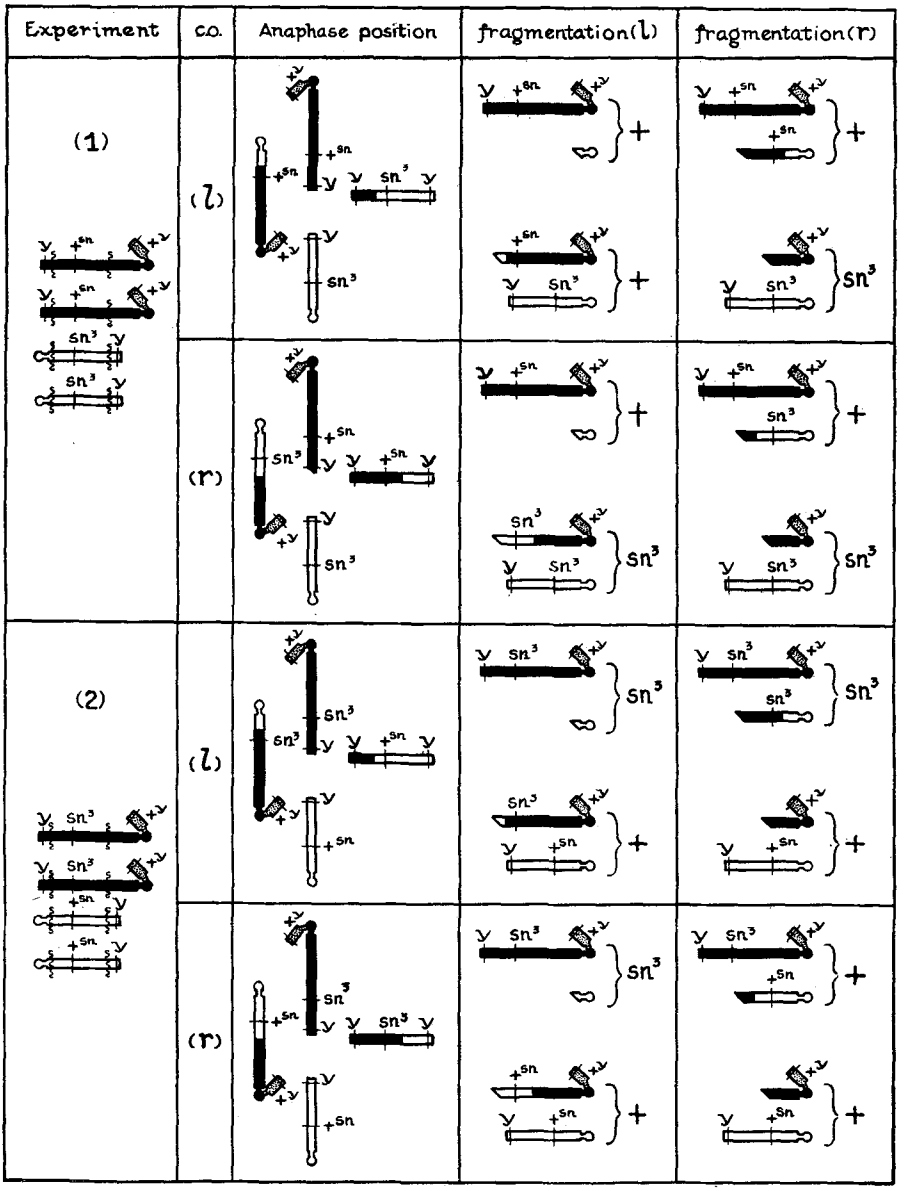


FIGURE 12. Two experiments involving *y*, *sn*^{3}, *bb*^{Df} and Theta (table 29a). The results of x-segregation after crossing over to the left (l) and to the right (r) of *sn*^{3} and of fragmentation of the chromatid with two fibre points to the left (l) and to the right (r) of *sn*^{3}. The lines } indicate the end points of the inverted region.

are fundamentally different. Whereas the numbers of different kinds of spots calculated as percentages of total number of spots in:

Experiment (1) of table 13 were: 80 *sn*^{3}; 13*y*; 3 *y*-*sn*^{3}; 4 others, the percentages in:

Experiment (1) of table 29a were: 54 sn^3; 22 y; 16 y-sn^3; 8 others.

More striking still is the difference between the experiments 2 without or with bb^{Df}:

Experiment (2) table 13: 88 sn^3; 11y; 1 other.

Experiment (2) table 29a: 4 sn^3; 85 y; 11 others.

These differences become intelligible if one takes account of the nature of bb^{Df} as representing both a deficiency for the inert region of the X chromosome and an inversion for most of the genetically active region. If we make the same assumptions regarding crossing over within the inverted section, segregation, and fragmentation as those used in the chapter preceding the last we arrive at the expectations presented in figure 12. It is apparent that only sn^3 spots will be produced (not considering at present that some of the + type segregates may lead to areas with male constitution). However, in experiment 1, 45 per cent of the spots were not-sn^3 and it is certain that even a large proportion of the 54 per cent sn^3 spots have to be regarded as rudiments of y and sn^3 twin spots. In experiment 2 the number of sn^3 spots deviates still more from expectation. Only 2 out of 49 significant spots were sn^3. Here the extreme rarity of sn^3 spots does not necessarily indicate an equal rareness of crossing over within the inversion. If the most frequent process of crossing over and fragmentation is represented by the process **r,r,** as was the case in certain earlier experiments, then no sn^3 spots will be produced by it in experiment 2, although they would appear in experiment 1 (fig. 12).

As crossing over within the inversion can at most account only for the occurrence of sn^3 spots, other processes have to be looked for to furnish an explanation of the y single spots and y and sn^3 twin spots. The small number of such spots recorded in table 13 had been shown to be due to somatic crossing over between the Theta-duplication and the homologous region of the right end (mainly) of the X chromosomes. If we consider the same process for the present experiments, we find the following expectations (fig. 13):

(a) Crossing over between Theta and an X chromatid of the chromosome not carrying bb^{Df} in experiment 1 leads to twin segregates with the constitutions y (3X)-sn^3 (1X, 2 Theta); in experiment 2 to twin segregates y (3X)-+(1X, 2 Theta).

(b) Crossing over between Theta and a bb^{Df} carrying X chromatid with equational **x** segregation in experiment 1 leads to y (3X)-+(1X, 2 Theta) twin segregates and in experiment 2 to y (3X)-sn^3 (1X, 2 Theta) twin segregates.

Thus crossing over involving Theta explains the occurrence of y spots. Will it also account for the y-sn^3 twin spots in experiment 1? It will do so under the assumption that the viability of 1X, 2 Theta segregates is

high enough to give rise to mosaic areas in a large number of cases. This assumption we had found justified during the analysis of experiments involving *y*, *sn*^{3}, *Mn*, and Theta. We can rule out the occurrence of crossing over process **b**, as it leads to *y-sn*^{3} twin spots in experiment (2) which did not occur. This failure in crossing over between Theta and the right end of the *bb*^{Df} chromosome is in agreement with the probably complete ab-

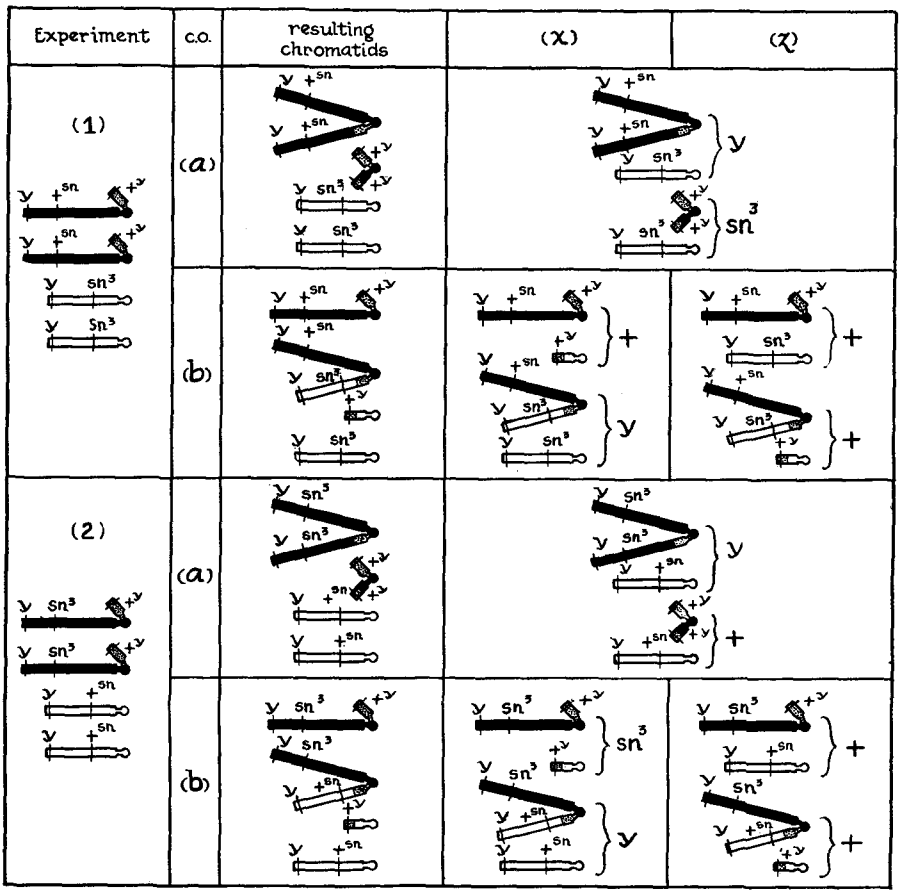


FIGURE 13. Two experiments involving *y*, *sn*^{3}, *bb*^{Df}, and Theta (table 29a). The results of crossing over between the homologous right regions of Theta and the X chromosomes and of subsequent **x**- and **z**-segregation.

sence of the homologous region in the deficient X chromosome. But if process **a** occurs in experiment 1 it accounts for the *y-sn*^{3} twin spots, the *y* single spots (as rudiments of twin spots), and an unknown proportion of the *sn*^{3} single spots (also as rudiments of twin spots). In experiment 2 it causes the *y* spots. It might be added that crossing over between the left end of Theta and the homologous left end of the X chromosomes yields *y* (2X) spots in both experiments and may contribute to this class.

An independent test of our explanation is provided by a consideration of the sex of spots (table 29b). In experiment 1, 35 *sn*^{3} spots had no male coloration, 7 showed male coloration, and in 34 cases a small spot of male coloration occurred near to the *sn*^{3} bristles. Singed-3 spots both with and without male coloration would be expected from crossing over within the inversion (fig. 12), according to the balance of sex factors brought about by the varying length of the duplicating fragment. In cases where crossing over within the inversion results in a *sn*^{3}, not-male-coloration segregate, the twin segregate will often have a constitution leading to $+^{sn}$, male-color areas. This may account for cases where a spot of male coloration was found near to *sn*^{3}, not-male-colored regions. (It is probable that some

TABLE 29b

Sexual coloration of critical spots from table 29a.

NO. OF EXP. IN TABLE 29a	*sn*^{3}		*y*		*y-sn*^{3}		+	*y-*
	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	♂ COL.	+♂ COL.
1	35	7; near 34	13	—	27	4; near 5	13	—
1′	3	—; near 2	1	—	3	—	—	—
2	—	—	6	—	—	—	4	2
2′	1(?)	—; near 1?	13	—	1	—	18	4

In addition exp. 2: 1 *y-sn*^{3}-+ ♀ col. triple spot.

of these cases were *sn*^{3} male-colored single spots in which the *sn*^{3} trichogenic cells have become isolated from the rest of the cells constituting the spot.) Finally, those *sn*^{3} spots which are rudiments of a *y* and *sn*^{3} twin segregate produced after crossing over between Theta and an X chromatid ought to be of male coloration. It is probable that some of the male-colored *sn*^{3} spots owe their origin to the last named process.

All *y* spots in experiment 1 were of "no male color" (13 cases), in agreement with their expected 3X (or sometimes 2X) constitution. A discrepancy, however, exists in the cases of *y-sn*^{3} twin spots. The hypothesis calls for *y* areas with not-male-coloration and *sn*^{3} areas with male constitution. Four such cases were found. Five more cases, in which a small spot of male coloration occurred near the twin areas may in reality be also of the expected *y* ♀, *sn*^{3}♂ kind. But in the majority of critical *y-sn*^{3} spots, (27 cases), no male coloration was visible in either twin area. The existence of this group raises doubts as to the validity of the present explanation. It is possible to make special assumptions to account for the unexpected type but they will not be given as proof is lacking.

The sex of spots in experiment 2 could be recognized in 8 *y* areas only. They were of not-male coloration in agreement with expectation and 2

were accompanied by an area of male coloration as demanded by segregation *y* (3X)-+(1X, 2 Theta).

No close analysis will be attempted for the observed single spots of normal setae with male coloration. Among other possibilities such spots are to be expected from crossing over within the inversion, and in experiment 2 as rudiments of twin spots from crossing over involving Theta.

If, in spite of the discrepancy pointed out, the general explanation given is accepted, then we come to the conclusion that the most frequent type of somatic crossing over is that between the right end of the Theta duplication and the homologous region of the not bb^{Df} bearing X chromatids. In the experiments of table 13 this process is rare but it is understandable that its relative frequency is high when bb^{Df} is present, for the following reasons. Normally the highest frequency of somatic crossing over occurs in the neighborhood of the fibre point. This region is abnormal in a bb^{Df} chromosome lacking most or all of its inert section as well as the *bb* locus and enclosing also one end point of the inversion. These conditions can be expected to interfere with pairing and crossing over of the right ends of the X chromosomes. But they should facilitate homologous pairing of the right end of the normal X chromosome with the homologous region of the Theta duplication.

Apart from the high relative frequency of certain types of spots the absolute frequency of mosaic areas is unusually high in comparison with the similar experiments of table 25a. It may be suggested that the presence of the Theta duplication is responsible for the difference. It contains inert material like a Y chromosome which, as shown above and again below, increases the total frequency of somatic crossing over.

No explanation can be given for the further difference between the frequencies in experiments 1 and 2 of table 29a.

Experiments involving y, sn^3, bb^{Df} and Theta; a Y chromosome present

The ability of a Y chromosome to increase the frequency of spots strongly is again demonstrated by a comparison in table 29a of experiments 1 and 2 with experiment 1′ and 2′. Under the influence of the Y chromosome the frequencies rise from 180 to 379 per cent and from 20 to 83 per cent. No change in relative frequencies of the different types of spots occurred nor did new types of mosaic areas appear. The coloration of the spots in regard to sex (table 29b) showed features similar to those brought out in the preceding section.

Apart from one discrepancy the experiments involving *y*, sn^3, bb^{Df}, and Theta with or without a Y chromosome seem to confirm the explanation proposed for similar experiments in which Theta was not present.

New facts were presented to show that somatic crossing over frequently

TABLE 30a

Spots in experiments involving primarily y, sn^3, Mn, bb^{Df}, and θ without or with a Y chromosome (see text).

EXP.	CONSTITUTION	IND.	SPOTS	%	sn^3 1	sn^3 2	sn^3 >2	sn^3 total	y 1	y 2	y >2	y total	$y\ sn^3$ 1	$y\ sn^3$ 2	$y\ sn^3$ >2	$y\ sn^3$ total	$y\text{-}sn^3$ 2	$y\text{-}sn^3$ >2	$y\ sn^3\text{-}sn^3$	+ >2	+♂ COL.	+♂ COL.-y 1	+♂ COL.-y 2	+♂ COL.-y >2
1	$\frac{y\ +\ +\ Mnbb^{D_s}}{y\ w^e\ sn^3\ +\ \theta}$	2685	666	25	35	11	97	143*	269	59	188	516†	2	—	—	2	—	3	—	2	—		—	
2	$\frac{y\ w^e\ sn^3\ Mn\ bb^{Df}}{y\ +\ +\ +\ \theta}$	320	207	65	5	3	2	10	110	33	41	184	1	—	—	1	4	6	—	—	2		—	
3	$\frac{y\ +\ Mn\ \theta}{y\ sn^3\ +\ bb^{Df}}$	28	75	268	11	11	23	45	4	2	1	7	3	5	2	10	4	4	5	—	—		—	
4a	$\frac{y\ w^e\ sn^3\ Mn\ \theta}{y\ +\ +\ +\ bb^{Df}}$ no Y	143	80	56	—	—	—		34	16	18	68	—	—	—		—	—	—	—	10	1	—	1
b	$\frac{y\ w^e\ sn^3\ Mn\ \theta}{y\ +\ +\ +\ bb^{Df}}$ Y	223	502	225	—	—	1(?)	1	140	86	166	392	—	—	—		—	—	—	—	71	10	10	18
c	$\frac{y\ w^e\ sn^3\ Mn\ \theta}{y\ +\ +\ +\ bb^{f}}$ Y	151	202	134	—	—	—		63	40	54	157	—	—	—		—	—	—	—	33	4	2	6

* 51 of these were clearly $+^M$ and 9 *Mn*.

† 71 of these were clearly $+^M$ and 19 *Mn*.

involves the Theta duplication and that the constitution 1X, 2 Theta is viable in hypodermal spots.

Experiments involving y, sn^3, Mn, bb^{Df}, and Theta

The conclusions arrived at in the last section are once more found to be in accordance with a series of experiments which contained *Mn* in addition to the genes present in the last group. The results (table 30a) are in marked contrast to those of table 16a, where no bb^{Df} chromosome was involved; but they are of the kind to be expected from the theory developed in the last chapter. These experiments were partly made before the role of the Y chromosome in somatic crossing over was known. From independent evidence, however, it is most likely that no Y chromosomes were present in experiments 1 and 2 while an extra Y chromosome probably was present in experiment 4b. In experiments 3 and 4a it is certain that the females inspected had no Y chromosome and experiment 4c was devised in order that a Y chromosome should be present.

TABLE 30b

Sexual coloration of critical spots from Table 30a and from similar experiments.

EXP.	sn^3		y		$y\ sn^3$	$y\ sn^3$-sn^3	+♂ COL.	y-+♂ COL.
	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	NO ♂ COL.	NO ♂ COL.		
1	1	39‡	—	—	—	—	—	—
2	—	—	23	—	—	—	2	—
3	5	3	—	—	1(?)	1*	—	—
4a	—	—	5	—	—	—	10	2
b	—	—	7	—	—	—	71	38
c	—	—	10	15(?)	—	—	33	12
$\frac{++\ Mn\ bb^{Df}}{y\ w^e\ +\ \theta}$	—	10	—	—	—	—	—	—
$\frac{y\ Mn\ \theta}{y\ +\ bb^{Df}}$ Y	—	—	2	1(?)	—	—	—	4

‡ Determination of sex based on abdominal coloration in 26 spots; on wing length in 2 spots; wing length and sex comb in 1 spot; w^e coloration in 10 spots.

* Determination possible in the sn^3 spot only.

A short analysis on the basis of our previous findings follows (table 31):

(a) Single crossing over within the inversion and followed by fragmentation of the two-fibre-point chromatid leads to sn^3 $+^M$ and + areas in experiment 1, to + and sn^3 *Mn* areas in experiment 2, and to sn^3 *Mn* areas in experiment 3. No mosaic areas are produced in experiment 4.

(b) Crossing over between Theta and the homologous right arm of a not-bb^{Df} chromatid leads to the following twin segregates:

Experiment 1: y(3X)-y *Mn* bb^{Df} (1X)

Experiment 2: y(3X)-y sn^3 *Mn* bb^{Df} (1X)

Experiment 3: $y(M/M/+;3X)$-sn^3 (1X, 2 Theta)

Experiment 4: $y(M/M/+;3X)$-+(1X, 2 Theta).

The 1X segregates of experiments 1 and 2 are expected to be inviable. Consequently *y* single areas will result in these two experiments. As hypodermal areas with three X chromosomes containing 2 Minute-n and one $+^{Mn}$ allele can be formed according to our earlier findings, *y* and sn^3 twin areas are expected in experiment 3 and *y* areas next to +-(1X)-areas in experiment 4.

TABLE 31

y, sn^3, *Mn*, bb^{Df}, θ

Types of segregates after different kinds of crossovers and fragmentation processes in experiments noted in table 30.

REGION OF C.O. AND FRAGMENTATION	EXP. 1	EXP. 2	EXP. 3	EXP. 4
l,l	sn^3 ·	+	dies	dies
	Mn	+	*Mn*	*Mn*
l,r	sn^3	+	dies	dies
	Mn	sn^3 *Mn*	sn^3 *Mn*	*Mn*
r,l	sn^3	+	dies	dies
	Mn	sn^3 *Mn*	sn^3 *Mn*	*Mn*
r,r	+	+	dies	dies
	Mn	sn^3 *Mn*	sn^3 *Mn*	*Mn*
c.o. θ-*X*	*y*(3*X*)	*y*(3*X*)	*y*(*M*/*M*/+)	*y*(*M*/*M*/+)
	dies	dies	$sn^3/\underline{\theta\theta}$	$+/\underline{\theta\theta}$

The actual findings lead to the conclusion that the majority of areas are derived from crossing over involving Theta. Although some of the following spots are probably produced by one or the other rarer processes, crossing over involving Theta can account for 516 out of 665 spots in experiment 1, for 184 out of 207 spots in experiment 2, for the *y* and sn^3 twin spots, and, as rudiments of twin spots, for the *y* single and an unknown number of sn^3 single spots in experiment 3, and in experiment 4a for all 80 spots namely the *y* and $+^y$ male-colored twin spots and as rudiments for the *y* single and $+^y$ male coloration single spots. The remaining spots can be derived from different processes of which single crossing over within the inversion accounts for the sn^3 spots in experiments 1 and 2 and for some of them in experiment 3.

In footnote ‡ to table 30a experiment 1 it is stated that of 90 *y* spots on head and thorax which were classifiable as to setae length, 71 were of approximately $+^M$ size and 19 were of M size. Crossing over between Theta and the right arm of the X chromosome leads to *y Mn*/*y*+/*y*+(3X)

spots which show about normal seta size. The *M* type spots therefore must have originated differently, for example, by crossing over between Theta and the homologous left part of the X chromosome. It is not possible to say whether the proportion of the two kinds of *y* spots in this head-thorax sample can or cannot be regarded as representative of the whole sample (see section on somatic segregation and ontogenetic pattern).

The determination of the sex of spots agrees on the whole with the expectation (table 30b). Attention may be directed to the fact that the sex of spots in experiment 1 could be judged not only by the coloration of abdominal spots but also by presence of sex combs, type of eosin eye color, and length of wing.

The absolute frequency of spots was high, even without the presence of a Y chromosome; 25 spots per hundred flies in experiment 1, 65 in experiment 2, 268 in experiment 3 and 56 in experiment 4a. Three of these values are, though somewhat higher, of the same order of magnitude as in experiment 2 of table 29a. The unusually high value of experiment 3 is similar to the one in experiment 1 of table 14. In both cases a *y sn*^{3} *bb*^{Df} chromosome was present but the cause of the unusually high percentage of spots is not apparent.

The presence of a Y chromosome, in experiments 4b and c raises the total frequency of spots from 56 to 225 and 134 per cent. Besides, in experiment 4c a certain number of unexpected *y* spots with apparently male coloration were found. Some of these most probably belong to the *y* (3X)-+^{y}(1X) twin group; but others were of *y* male coloration indeed. Perhaps segregation similar to that found in the exceptional experiment with *y bb*^{Df}/*sn*^{3}; Y occurred here.

Information on experiments with *y Mn bb*^{Df}/*y w*^{e} and *y Mn θ*/*y bb*^{Df} Y flies is added in table 30b. The results agree with the rest.

On the whole the theory of somatic crossing over in flies heterozygous for an X chromosome inversion has stood the exacting test provided by the experiments with *y*, *sn*^{3}, *Mn*, *bb*^{Df}, and Theta. If the theory is accepted, the present section adds weight to the deduction that two doses of *Mn* in triplo-X are not lethal to hypodermal areas. This will be of importance for the interpretation of mosaics in flies with a ring-shaped X chromosome (X^c).

Experiments involving the dl-49 Inversion

An X chromosome inversion has been involved also in certain experiments which were discussed earlier. This is the "dl-49" inversion (tables 5 and 13). It is a shorter inversion than the one associated with *bb*^{Df}, extending only from locus 11± to 42±. Consequently all crossovers from 42± to the right end are outside of the inversion; such crossovers formed

the basis of the discussion. Crossing over within the inverted region leads to sn^3 spots and it is probable that some of the observed sn^3 spots owe their origin to such crossover types.

THE INFLUENCE OF A Y CHROMOSOME ON MOSAIC FORMATION IN FEMALES NOT CARRYING A bb^{Df} INVERSION

Only one experiment with flies containing two X chromosomes without an inversion gives information on the influence of a Y chromosome under these conditions. It concerns the flies of experiment 2, table 10 which have been discussed before. The female parents of these individuals were XXY so that they themselves consist of XX and XXY flies in about equal numbers. The total frequency of spots in these flies was 72.8 per cent as opposed to 3–12 per cent in experiments 1, 3, and 4 of the same group. The rise in frequency is presumably due to the presence of the Y chromosome in half of the flies. The influence of the Y is of course even greater in this experiment than the number 72.8 per cent indicates, for this represents the average percentage for all individuals of experiment 2, about one-half of which were of XX constitution.

In table 15 it had been shown that 9 out of 11 spots of experiments 1 and 4 of table 10 were not male-colored. However, 17 out of 30 spots in experiment 2 showed male coloration (table 32). According to the simplest

TABLE 32

Sexual coloration of critical spots on y Mn/sn³ flies without or with a Y chromosome (cf. table 10, experiment 2).

TYPE OF SPOT	*y*			*sn³*				*y-sn³*			+♂ COL.
	NO ♂ COL.	♂ COL. NEAR		NO ♂ COL.		♂ COL. NEAR	♂ COL.	NO ♂ COL.	♂ COL.		
Number	2	3		9‡		5†	1*	2	1		7
Y constitution of flies	?	?	XXY	XX	?XXY	?	XXY	XXY	?	?	?	XXY
Number	2	1	2	1	7 1	1	4	1	2	1	5	2

Totals: not male-colored, 13; male colored, 17.

‡ Doubtful: 2 spots.

† Doubtful: 3 spots.

* Doubtful: 1 spot.

form of the theory of somatic crossing over and segregation, no spots with male constitution were expected in these flies. The presence or absence of a Y was tested in 25 cases, the test consisting of a determination of the frequency of non-disjunctional sons and their fertility or sterility. Fertility indicates presence of a Y in the mother, sterility its absence. Lack of exceptional sons does not prove that the mother had no Y chromo-

some but may only be due to too small a total progeny. This test method cannot serve as more than a first survey.

After disregarding the 5 individuals (representing 3 not-male-colored and 2 male-colored spots) which could not be tested there were left 10 not-male-colored and 15 male-colored spots. Only one of the 10 not-male-colored spots could be shown to have occurred on an XXY individual, while 9 out of the 15 male-colored spots were proved to have been on XXY females. The difference between these two distributions is significant ($\chi^2 = 6.25$; P between 0.01 and 0.02). We can conclude that the presence of a Y chromosome increases the number of spots of male coloration in females which do not carry an X chromosome inversion or duplication. The data are not conclusive as to the question whether male-colored spots in such females occur *only* if a Y chromosome is present, since the constitution of the 6 individuals with male-colored spots is not known. They may have been either XXY flies which did not produce exceptions or all or some of them may have been XX. In other experiments at least one case of a male-colored spot was found which occurred in a female in which certainly no Y chromosome was present. This female was supposedly perfectly normal in its chromosomal constitution, but the possible presence of a duplication of new origin cannot be excluded.

The action of the Y chromosome in the experiment just reported reminds us of the numerous male-colored spots in the exceptional experiment with *y bb*^{Df}*/sn*^{3}; Y females. There we demonstrated how somatic crossing over in XXY females between an X and the Y may give rise to XX and XXY twin spots, the latter constitution being responsible for the male coloration. It is possible that the occasional male spots found in several of our experiments (table 15, lines 1–3, and others) are due as a rule to the unsuspected presence of a Y chromosome.

If further experiments show that this is true, an at least partial explanation would be available for the fact that BRIDGES (1925) found the sex of spots in his *Mn* individuals to be always male, while most of our experiments with flies of similar constitution yielded mainly female spots. BRIDGES reports that the frequency of spots in his cultures was between 10 and 40 per cent. Considering that BRIDGES paid predominant attention to larger spots, these frequencies are very high. If we assume that supernumerary Y chromosomes were present in BRIDGES' stocks, we have an explanation both for the high frequencies of spots and for their male constitution.

MOSAICS IN FLIES HETEROZYGOUS FOR A RING-SHAPED X CHROMOSOME

Flies heterozygous for a ring-shaped "closed X chromosome" have been reported to have yielded numerous gynandromorphs (L. V. MORGAN,

TABLE 33a

Spots in X^c flies.

Experiments 2–4, 3, 9, 12–14 were inspected with low power only, therefore mainly head-thorax macrochaetae are involved.

EXP.	CONSTITUTION	IND.	SPOTS	sn^3 or f^5			y			$y\ sn^3$			+			y-sn^3 (or f^5)		OTHER SPOTS*
				1	2	>2	1	2	>2	1	2	>2	1	2	>2	2	>2	
1	$\frac{y\ X^c}{sn^3}$	153	147	100	12	19	11	2	2							—	1	
2	$\frac{y\ X^c}{sn^3}$	289	25	22	1	1	1	—	—									
3	$\frac{y\ X^c}{y\ sn^3\ bb}$	134	26				1	—	—	23	1	1						
4	$\frac{yX^c}{y\ sn^3\ bb}$ (XX & XXY)	483	59							58	—	1						
5	$\frac{y\ X^c}{w^{bf} f^5}$	—	109	67	9	5	21	—	3							2	1	1
6	$\frac{y\ X^c}{ysn^3\ \theta}$	97	97	71	6	10	5	—	—							2	3	
7	$\frac{y\ X^c}{(w)\ f\ Mn}$	45	47	—	—	1	—	—	3				14	2	26			1
8	$\frac{y\ X^c}{sn^3\ Mn}$	107	151	77	11	5	6	1	2				19	—	26	—	3	1
9	$\frac{y\ X^c}{ywMn\theta}$	27	15										7	—	8			
10	$\frac{y\ sn^3\ X^c}{+}$	—	27	4	—	—	3	—	1	14	1	3						1
11	$\frac{y\ sn^3\ X^c}{w\ bf\ f^5}$‡	110‡	57	11	3	11	2	—	1	17	1	6						5
12	$\frac{y\ sn^3\ X^c}{wmf\ Mn}$	58	35	1	—	—				1	—	3	20	2	6			2
13	$\frac{y\ sn^3\ X^c}{\text{Bld}\ w}$ (Bld-Minute)	—	20				6	—	2	8	2	2						
14	$\frac{y\ f\ Mn\ X^c}{sn^3}$	55	45	27	2	14							1	—	1			

‡ In this series most single microchaeta spots were not recorded on account of the uncertainty as to whether they were sn^3 or f^5. The spots listed include mainly macrochaetae spots. Those given in the first column are believed to represent f^5.

* Phenotype of these spots:

Experiment 5: +♂ col.

Experiment 7: +♂ col.

Experiment 8: y-$y\ sn^3$-+♂ col. triple spot (?).

Experiment 10: y (?)-$y\ sn^3$ twin spot.

Experiment 11: $y\ sn^3$-f^5 twin spots: 3-y(?)-$y\ sn^3$ twin spot: 1.-y(?)-$y\ sn^3$-f^5 triple spot: 1.

Experiment 12: $+^y+^M$ (f or $+^f$?).

1926, 1933). At a stage of this investigation when the relation of mosaic occurrence to somatic crossing over had not yet been recognized, varied experiments with this closed X chromosome, which hereafter will be referred to as X^c, were undertaken in order to clear up the problem of origin of mosaic areas. It was found that the frequency of gynandromorphs in flies heterozygous for X^c was not appreciably higher than usual. This had also been true in the later experiments of Mrs. MORGAN and it must be concluded that the original high incidence of gynandromorphs was not due to the closed X condition by itself, but due to either accessory or independent causes which have ceased to exist in the stock.

Although gynandromorphs were practically absent, a high frequency of spots of different kinds was encountered. The data are assembled in table 33a. In order to simplify the presentation, no distinction has been made as to the determination of the seta length in the different groups of spots. In most cases where only microchaetae were involved, no such determination was possible. However, when macrochaetae were included in spots it was seen that they fell into two groups, spots with about normal sized setae and spots with very small setae. A total of 219 spots could be thus classified according to bristle size. Often in cases of sn^3 or f^5 setae no finer distinction as to size was possible. Among the class $+^M$ it was found that generally the size of bristles was smaller than normal although distinctly larger than *Mn*.

In many cases there was a high correlation between the length of setae and the area covered by spots. Thus the total number of small and normal sized bristles in the sn^3 or f^5 group was 67 small and 4 normal among the single-setae spots, while among spots comprising more than one seta all 15 cases which could be classified as to seta size had setae of normal length. Among the *y* spots the relation is: 6 small and 8 normal single-bristle spots and 4 normal sized larger-area spots. Here no correlation is apparent for reasons which will become clear later. Among the *y* sn^3 spots were found: 105 single-seta spots with small and 2 with normal sized setae; larger spots—1 with small, 2 with normal sized setae.

It is immediately apparent that no simple hypothesis of elimination of a chromosome can explain the types of spots found. Such an hypothesis cannot account, among other facts, for the following:

(a) Appearance of numerous $+^M$ spots in experiments 7, 8, 9, and 12. If the *Mn* carrying chromosome were eliminated, *y* or sn^3 spots would be expected, if the not-Minute carrying chromosome were excluded, the remaining constitution would be lethal.

(b) Appearance of *y* and of sn^3 spots in experiments 10 and 11 where the original chromosomes carried both *y* and sn^3 or neither.

(c) The fact that 23 out of 55 critical spots were not of male constitution.

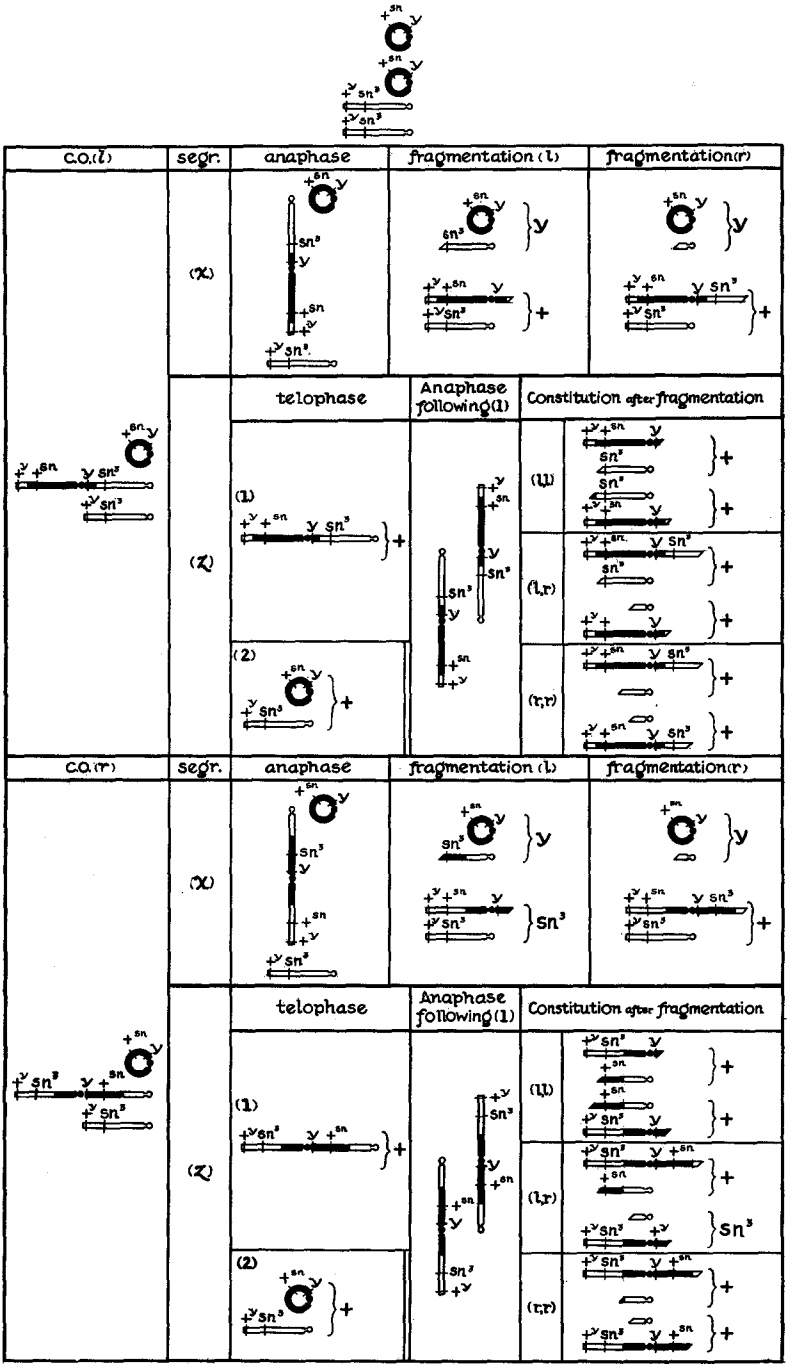


FIGURE 14. *y* X^c/sn^3. The results of crossing over to the left (l) and to the right (r) of sn^3. Subsequent x-segregation accompanied by different types of fragmentation of the two-fibre-point chromatid to the left (l) or right (r) of sn^3 or subsequent z-segregation followed, in one segregate, by different types of fragmentation (l, l; l, r; r, r) of both two-fibre-point chromatids.

(d) The appearance of abnormally short setae.

The occurrence of different types of spots and the relation between size of setae and amount of area covered by the spot become understandable under the assumption of somatic crossing over and segregation and taking account of the cytological peculiarity of the X^c chromosome, as will be shown now.

In examining the consequences of somatic crossing over we shall restrict ourselves to cases of single crossovers. The analysis will be given here in detail for one case only, represented by the experiments 1 and 2 and concerning flies with the constitution $y\ X^c/sn^3$ (fig. 14). Experiment 5 with $y\ X^c/w^{bf}\ f^5$ flies is very similar.

Crossing over between one strand of the ring chromosome and a strand of the normal X chromosome results in a long, open chromatid consisting of two full X chromatids with two fibre attachment points. This chromatid possesses one sister fibre point of the normal X chromosome on one end and one sister point of the ring chromosome in the middle. A double chromatid of this type has been called a "tandem" by L. V. MORGAN. If separation of sister points proceeds normally, two types of segregation have to be considered:

(1) **x**-segregation. The non-crossover chromatids go to opposite poles, the tandem being subjected to opposite forces on account of its two fibre points tending in different directions. This may result in either **(a)** non-inclusion of the tandem into the daughter nuclei and subsequent elimination, or in **(b)** fragmentation of the tandem between the two fibre points and inclusion of the two fragments within the two daughter nuclei. (A third possibility, the inclusion of the whole tandem chromosome into one daughter nucleus is least probable and will not be discussed.) Process **a** leads to *y* and sn^3 twin segregates with 1X constitution, process **b** to different pairs of segregates depending on the locus of crossing over and of fragmentation: **(l,l), (l,r)** and **(r, r)** to *y* and + twins and **(r,l)** to *y* and sn^3 twins. While the segregates from **a** are both of male constitution, those of **b** contain a deficiency or a duplication for a left section of an X chromosome. Their sex is dependent on the extent of the deficiency or duplication.

(2) **z**-segregation. The non-crossover chromatids go to one pole, the tandem chromatid to the other pole. In this case the two segregates will be of the same genic constitution as the rest of the fly. However, the tandem-containing segregate will divide and its halves undergo fragmentation during the next cell division. The position of the two sister tandem-chromosomes is expected to be of such a nature as would result from sister fibre points going to different poles. Three combinations of fragmentation in the two separate tandems can occur: breaks in both to the

left of sn^3, in both to the right, and in one to the left and one to the right. Unless the two tandems happen to break at identical places unbalanced segregates will result. Their phenotype is in most cases + except in one in which a sn^3 segregate is produced.

When we compare these expectations with the actual results it becomes clear that the process **x** can at best account for only a small number of spots.

(1a) If the tandem were eliminated completely, *y* and sn^3 twin spots with male coloration should appear. Only one twin spot occurred; the proportion of *y* and sn^3 single spots is so unequal that only a few of them can be regarded as vestiges of twin spots. In addition, neither the fact that 11 out of 19 critical spots were not of male constitution nor the small size of most setae in single seta sn^3 spots agrees with the assumption of complete elimination.

(1b) Should the tandem undergo fragmentation during **x**-segregation we would meet a similar lack of agreement between facts and expectation. In three out of the four cases *y* spots will be produced, while *y* and sn^3 segregates result from the fourth. The observed *y* spots are regarded as results of **x**-segregation and fragmentation, but the great majority of spots are single sn^3 spots. Even if one assumes that the unbalanced *y* segregate of the twin cells is inviable, so that only single sn^3 areas are produced, the expectations as to bristle size would be mainly for normal length. For the sn^3 segregate would contain two complete X chromosomes and in addition a duplicating X fragment; such hyperploid condition leads to strong setae (PATTERSON, STONE and BEDICHEK 1935).

(2) The majority of spots can be understood when we assume **z**-segregation to have preceded them. The only segregate resulting in a visibly aberrant area is of the phenotype sn^3. Furthermore it contains only one complete X chromosome besides two fragments which together represent less than a second X chromosome. The sections represented by the two fragments are variable, depending on the locus of fragmentation. Such hypoploid conditions may be expected to result often in short-bristled hypodermal cells of low viability. The 122 sn^3 single-seta spots of experiments 1 and 2 and the 67 f^5 single-seta spots of experiment 5 correspond to this type.

To account for the smaller number of sn^3 spots which are of larger size and possess bristles of normal length one can assume both that some of them represent special hypoploid conditions due to **z**-segregation which are favorable in respect to genic balance and also that they are results of double crossing over to both sides of sn^3, a process which will lead to normal segregation of two and two X chromosomes and homozygosity for sn^3 in one segregate.

The sexual coloration of critical spots in experiments 1 and 5 was male in 9, not-male in 10 sn^3 spots and as regards y spots male in 1 and not-male in 2 cases (table 33b). No very definite expectation on the basis of the proposed theory is possible, since hypoploid conditions may lead to both types of sexual characteristics (which involve intersexual conditions) according to the length and region of the duplicating fragments involved. Those sn^3 spots which are derived from double crossovers are expected to be of normal female constitution.

TABLE 33b

Sexual coloration of critical spots from table 33a.

	sn^3 OR f^5		y		$y\ sn^3$					
	NO ♂	♂	NO ♂	♂	NO ♂	♂	+♂	y-sn^3 ♂	$y\ sn^3$-f^5 ♂	$y\ sn^3$-y(?) NO ♂
1	9	8	2	—						
5	1	1	—	1			1			
6	3	4						1†		
7				1*			1			
8	4	1						1		
10					1	—				
11		6				1			2	1
14	1	2								
	18	22	2	2	1	1	2	2	2	1

Totals: not male colored, **22**; male colored **31**.

† Determination of sexual coloration only possible in sn^3 area.

* Sex comb.

If one applies the theory of somatic crossing over, segregation, and fragmentation of the tandem chromosome to the other experiments reported in table 33, one arrives at specific expectations in each case with regard to the major types of spots. The derivations have to take account of the different regions of the chromosomes in which crossing over and fragmentation can occur and are rather lengthy in some cases. The resulting expectations are summarized in table 34 together with the observed facts. There is a good agreement both as to kind of main spots and as to the larger frequency of spots produced in consequence of **Z**-segregation as opposed to **X**-segregation. One exception with respect to the latter point is found: In experiment 10 the number of $y\ sn^3$ spots exceeds that of sn^3 spots, although the former are expected from **X**- and the latter from **Z**-segregation. Such a case probably would lose its peculiar character if one could adjust the expectations to the supposedly different frequencies of crossing over in different regions. But no attempt toward a finer analysis of the data presented will be made here. Such an analysis should include addi-

tional facts to be derived from the use of the newly discovered closed X chromosome which carries the normal allele of yellow.

TABLE 34

Expected and observed spots in experiments with X^c flies.

EXP.	CONSTITUTION	EXPECTATION‡		OBSERVED SPOTS	
		(z)	(x)	LARGEST CLASS	2ND LARGEST* CLASS
1, 2, 5	$\frac{y\ X^c}{sn^3 \text{ or } f^5}$	sn^3	y, y-sn^3	sn^3	y
3, 4	$\frac{y\ X^c}{y\ sn^3\ bb}$ (Y)	$y\ sn^3$	$y\ sn^3$	$y\ sn^3$	—
6	$\frac{y\ X^c}{y\ sn^3\ \theta}$	sn^3	sn^3	sn^3	—
7, 8	$\frac{y\ X^c}{sn^3\ Mn \text{ or } w\ f\ Mn}$	$+^M$, sn^3	y, y-sn^3	$+^M$	—
9	$\frac{y\ X^c}{y\ w\ Mn\ \theta}$	$+^M$	$+^M$	$+^M$	—
10	$\frac{y\ sn^3\ X^c}{+}$	sn^3	$y\ sn^3$, y	$y\ sn^3$	sn^3, y
11	$\frac{y\ sn^3\ X^c}{w^{bf}\ f^5}$	f^5, sn^3	$y\ sn^3$, y, $y\ sn^3$-f^5 etc.	f^5	$y\ sn^3$
12	$\frac{y\ sn^3\ X^c}{w\ m\ f\ Mn}$	$+^M$, sn^3	$y\ sn^3$, y	$+^M$	—
13	$\frac{y\ sn^3\ X^c}{\text{Bld } w}$ (Bld-Minute)	y, $y\ sn^3$	$y\ sn^3$, y	$y\ sn^3$	y
14	$\frac{y\ f\ Mn\ X^c}{sn^3}$	$+^M$, sn^3	$+^M$, sn^3, y-sn^3	sn^3	—

‡ Expectation in case of y, sn or f not specified in respect to M or $+^M$.
* Only given if larger than 10% of largest class.

There was an opportunity, in experiment 4, to discover a possible influence of the Y chromosome on the occurrence of spots. Part of these flies carried a Y chromosome, but no effect became apparent.

In addition to the demonstration that the theory of somatic crossing over can give an explanation for spots in flies with a closed X chromosome a new result is contained in these experiments. This is the relative frequency of **x**- and **z**-segregation. While in the experiments discussed in

former sections of this paper **z**-segregation does not lead to mosaic areas and its frequency therefore cannot be directly determined, in the present cases it occurs far more often than **x**-segregation. It appears probable that this is a consequence of particular conditions brought about by the presence of the tandem chromosome. These seem to result in preferential chromatid segregation, so that the two non-crossover strands go most frequently together to one pole and the tandem with its two fibre points to the other pole.

Changes in ring-shaped chromosomes during somatic divisions have been demonstrated cytologically by McCLINTOCK (1932) in maize. She has pointed out that somatic crossing over is probably responsible for these changes and that they are correlated with the origin of mosaics.

SEX-LINKED MOSAIC AREAS IN SUPERFEMALES AND IN MALES

No special study of sex-linked spots in flies of not-female constitution was made but some incidental observations seem worth recording.

Superfemales (3X+2A)

(1) *The three X chromosomes free.* Of 11 superfemales of the constitution *y sl*^{2} *bb*^{Df}/*y sn*^{3}*bb*/*sn*^{3} inspected, 3 were free from spots, while the remaining flies exhibited 18 *y* spots (1 seta: 12 spots; 2 setae: 3 spots; >2 setae: 3 spots). In five other flies of the same constitution 5 *y* spots and 1 *y* and *sn*^{3} twin spot (2 setae) were found.

(2) *Two attached X chromosomes, one free*: 14 $\widehat{yy}$/*sn*^{3}*bb* individuals possessed 3 *y* spots (1 seta: 2 spots; 2 setae (no ♂ col.): 1 spot); 19 $\widehat{yy}$/*y sn*^{3}*bb*^{Df}(Y?) individuals possessed 4 *y sn*^{3} spots (1 seta: 1; 2 setae: 10; >2 setae: 2).

The interpretation of these spots according to the theory of somatic crossing over is obvious.

Males

Spots involving segregation of sex-linked genes generally cannot be discovered in males. The only possibility which should give visible mosaic areas would be a segregation of two sister X chromosomes into one nucleus and resulting in a female spot. No case of this kind was encountered but the available data are not extensive enough to exclude the occurrence of such spots.

The situation is different in case of presence of an X chromosome duplication. Males which, besides a *y sl*^{2} *bb*^{Df} X chromosome and (most probably) a Y chromosome, possess a separate chromosome consisting of a Theta duplication attached to the short arm of a Y chromosome have not-yellow body color and setae. They show *y* spots with *y* setae very frequently. This would be understandable if somatic crossing over between

homologous parts of the Theta chromosome and the Y chromosome and normal segregation occurs.

It should be added that 65 males of the constitution "*y sn*^{3}; Theta, Y^s; no free Y" did not exhibit spots; nor did 135 "*y*, Theta; Y" and 85 "*y sn*^{3}, Theta; Y" males.

RELATIONS BETWEEN SOMATIC CROSSING OVER AND THE ONTOGENETIC PATTERN

The frequency of somatic crossing over is dependent on different factors. An influence of the environment was shown by BRIDGES (MORGAN, STURTEVANT and BRIDGES 1929) who found a decrease in number of spots with the progressing age of the culture, independent of the age of the mother. Experiments in our laboratory demonstrate an effect of varying temperature (STERN and RENTSCHLER 1936). Genetic factors which influence the percentage of spots are the Minutes, an extra Y chromosome, and probably different other factors, as judged from the variability of our results.

The dependence of the frequency of spots on different agents results in the appearance of different types of mosaicism. We thus have a parallel to the variable frequencies of piebald areas in mammals or to mosaic conditions in many organisms in general. It is possible to carry the comparison further. It can be shown that not only the frequency but the size and distribution of spots over the body is variable and dependent on different agents. This means that the time and frequency of the origin of segregates is independently variable in different regions of the developing organism. That this is true for mosaics which have been ascribed to mutations of unstable genes is well known (DEMEREC 1935). The following gives a corresponding account for spots which are known to be due to somatic crossing over.

With regard to differences *in time* of occurrence we shall refer only to two earlier statements. It was found (1) that the proportion of "1-seta spots" to "larger than 1-seta spots" is different in different experiments (table 3; see also other tables) and (2) that left crossovers are represented by large and small spots in some experiments (table 8) while they result only in single-seta spots in others (tables 10, 12). The causes for the variability in time of origin of spots are not known for the experiments referred to under (1). As to (2), it seems that the presence of *Mn* is correlated with the shift in occurrence of double crossovers.

The dependence of incidence of somatic crossing over on the spatial ontogenetic pattern, that is, on the conditions offered by different body regions of the larvae will be illustrated by a series of results. Such a dependence first became evident from the analysis of table 3, where the

TABLE 35

Distribution, in percentage of total, of spots over head, thorax, and abdomen in different experiments.

CONSTITUTION	NO. OF SPOTS	HEAD	THORAX	ABDOMEN
sn^3 Mn/y g^2 ty	120	5	58	37
y/sn^3	212	4	43	53
y Mn bb^{Df}/y w^e sn^3 θ	666	4	36	60
y w^e sn^3 Mn θ/y bb^{Df}	80	1	3	96
y w^e sn^3 Mn θ/y bb^{Df} Y	203	0	1	99
Setae Inspected*	—	4	30	66

* In this and in the following two tables the term "setae inspected" refers to the percentage of all inspected setae which were located on the respective body regions. For detailed account see "Methods" and table 1.

average increase of number of spots in Minute flies as compared with not-Minute flies was lower for the abdominal regions than for the head and thorax or from the fact that the proportion of single-seta spots to larger spots was lower in the abdomen than in the head-thorax region (table 3). Other examples of greatly varying distribution of spots over the three main body regions are given in table 35. While this table is concerned with total number of spots, a more detailed analysis can be derived from a separate consideration of different types of spots (table 36). In experiment 1 no different distribution of sn^3 single and y and sn^3 twin spots is expected, because nearly all sn^3 single spots are regarded to be rudiments of twin spots. This expectation is fulfilled, as the last columns indicate. With re-

TABLE 36

Distribution, in percentage, of different types of spots over head, thorax, and abdomen.

CONSTITUTION	SPOTS		HEAD	THORAX	ABDOMEN	χ^2	P
	TYPE	NO.					
(1) y/sn^3	y	59	10	48	42	y, twin: 9.614	<0.01
	sn^3	82	2	35	63	y, sn^3: 8.186	0.01–0.02
	y-sn^3 twin	67	0	40	60	sn^3, twin: 2.101	0.03–0.05
(2) y bb^{Df}/sn^3	y	109	0	16	84	y, sn^3: 2.203	0.01–0.02
	sn^3	60	0	8	92		
(3) y sn^3 bb^{Df}/y θ	y	151	0	8	92	y, sn^3: 23.321	<0.01
	sn^3	382	0	1	99	y, twin: 6.885	<0.01
	y-sn^3 twin	113	0	1	99	sn^3, twin: 0.020	> .99
(4) y Mn bb^{Df}/y w^e sn^3 θ	y	497	2	33	65	y, sn^3: 14.614	<0.01
	sn^3	134	8	34	57		
Setae Inspected		—	4	30	66		

spect to the distributions of *y* single and *y sn*^{3} twin spots as well as for that of *y* and of *sn*^{3} single spots, the χ^2 test points to significant differences. The majority of *y* spots represent rudiments of twin spots and should be distributed in the same way as the *sn*^{3} single and the twin spots. Therefore the deviation is interpreted to mean that those *y* spots which owe their origin to crossing over between *y* and *sn*^{3} have a distribution which is distinguished from the rest. The data indicate that the head and thorax regions are more favorable to left crossovers than to crossovers near the fibre point.

TABLE 37

Distribution, in percentage, of different spots over the abdominal tergites 1–7.

CONSTITUTION	SPOTS		ABDOMINAL TERGITES						χ	P
	TYPE	NO.	1+2	3	4	5	6	7		
y/*sn*^{3}	*y*	24	4	25	21	29	21	0	*y*, *sn*^{3}: 1.403	0.8– 0.9
	sn^3	51	4	22	33	24	17	0	*y*, twin: 8.787	0.05–0.1
	y-sn^3 twin	40	10	25	33	17	15	0	*sn*^{3}, twin: 1.861	0.7 –0.8
y bb^{Df}/*sn*^{3}	*y*	55	5	13	32	41	9	0	*y*, *sn*^{3}: 16.390	<0.01
	sn^3	91	2	40	29	27	2	0		
y sn^3 *bb*^{Df}/*y θ*	*y*	139	19	18	22	30	11	0	*y*, *sn*^{3}: 20.590	<0.01
	sn^3	376	4	21	20	29	25	1	*y*, twin: 13.277	<0.01
	y-sn^3 twin	112	4	22	20	32	22	0	*sn*^{3}, twin: 0.8417	0.5–0.7
y Mn bb^{Df}/*y w*^{e} *sn*^{3} *θ*	*y*	323	13	30	32	21	4	0	*y*, *sn*^{3}: 35.0392	<0.01
	sn^3	77	5	23	21	30	20	1		
Setae Inspected		—	10	13	17	24	24	12		

No significantly different distribution of the two types of spots is apparent in experiment 2. Both types result mainly from single crossovers within the inversion. An analysis is impeded by the possibility that different body regions may conceivably be variable in their effect on the viability of hypo- and hyperploid cells and thus lead to a differential survival of spots. This factor may play a role also in the other experiments to be discussed.

In experiment 3 no significant deviation of the distribution of *sn*^{3} single and twin spots occurred, although these spots owe their origin largely to different processes. However, the distribution of *y* spots differs from both that of *sn*^{3} and that of twin spots. While the first deviation is not surprising, since the two kinds of spots are mainly results of different crossovers, the distribution of *y* single and of twin spots should be identical, for the *y* spots are regarded to be rudiments of twin segregates. Possibly viability differences of the *y* and *sn*^{3} twin segregates in different body regions are responible for the result.

In experiment 4 the distributions again differ significantly. Dependence of the occurrence of the two kinds of crossovers or of the viability of segregates upon conditions in different body regions seem to be involved.

The same four experiments which were considered with regard to head-thorax-abdomen distribution of spots have been analyzed as to location of spots on the tergites of different abdominal segments (table 37). In experiment 1 no significant deviation in distribution occurred between *y* and *sn*^{3} single spots nor between *sn*^{3} single and twin spots, while the *y* single and the twin spots were probably significantly different. Taking

TABLE 38a, b

Distribution of y and sn^3 *spots over different body regions. Only spots from* $yMnbb^{Df}/yw\ sn^3\ \theta$ *flies of tables 36, 37 are included which occurred on individuals bearing at least one of each kind of spot.*

38a

SPOTS		HEAD	THORAX	ABDOMEN
TYPE	NO.			
y	29	2	6	21
sn^3	37	2	16	19

38b

	ABDOMINAL TERGITES				
NO.	1+2	3	4	5	6
21	4	7	4	4	2
19	1	5	3	6	4

account of the smaller numbers, the earlier comments on this experiment seem to apply here likewise. In experiment 2 the *y* and *sn*^{3} spots are doubtless distributed differently on the abdomen. In experiment 3 the similarities and differences resemble those of the distributions over head-thorax and abdomen. Lastly, in experiment 4, a striking dependence of type of spot on the abdominal region is apparent, showing a peak in frequency for *y* spots on the third and fourth segments and for the *sn*^{3} spots on the fifth segment.

It might be asked whether the differential distribution of different kinds of spots may be due to their occurrence on different individuals. To answer this question tables 38a and b are presented, in which are included all those *y* and *sn*^{3} spots of experiment 4 which occurred on individuals bearing at least one of each type. The numbers are too small to give significantly different distributions. However, in the abdominal series it is evident that the trend of frequencies for the *y* and *sn*^{3} spots coincides well with those of table 35, experiment 4.

The interpretation of the observed differences in abdominal distribution has to take account again of both differences in incidence of crossovers and of viability values. But it seems improbable that the survival values of different segregates vary enough in different tergites to cause the observed patterns by themselves. The participation of the body pattern as differ-

ential in regard to occurrence of somatic crossover types is regarded to be a contributing if not the main factor.

A comparison of the observed distribution of frequencies of spots with the numbers of setae inspected in different body regions (see last line of tables 35–37) shows that no random distribution took place. This is a further demonstration of the influence of body pattern on crossover occurrence. A finer analysis will depend on detailed knowledge and comparison of the developmental events within the different imaginal discs.

DISCUSSION

The evidence presented in this paper shows that mosaic areas on the body of *Drosophila melanogaster* appear in a varying percentage of flies whenever they are heterozygous for genes whose homozygous effect is recognizable in small spots. Theories as to the causation of these spots have to be based on somewhat intricate deductions. The observable phenomenon is limited mainly to yellow or singed single spots, or yellow and singed twin spots, in different proportions and with a few other attributes such as sexual coloration or seta length. The test of any theory as to the mechanism of spot production has to consist in its application to a varied group of genic combinations. Such a test is able to exclude definitely theories which cannot account for the actual facts. But if it succeeds in giving a satisfactory basis for them, it cannot claim a final "proof." In our special case we can say that the theory of chromosome elimination has been shown definitely unsatisfactory. The validity of the theory of somatic crossing over and segregation rests on its faculty to explain the manifold results presented in this paper. Throughout the text the word "assumption" has been used freely in order to leave no doubt as to the procedure of deduction. But it should be noted that most of these assumptions are justified from other experience.

There is a higher degree of safety in the discussion of somatic crossing over in cases where the X chromosomes were normal than in those where inversions or the closed X chromosome were involved. In these latter cases the claim is made that the proposed theory is able to account for most facts (some notable discrepancies have been pointed out), but the possibility of inventing other schemes should be stressed.

This analysis of somatic crossing over can by no means be regarded as complete. It was restricted in most cases to a consideration of single crossovers, although the rarer occurrence of double crossovers has been ascertained. No attempt was made to discuss multiple crossing over in detail, as for instance the relation of the different crossovers to different chromatids, or questions of interference. Neither has the probability been dealt with that somatic crossover processes may occasionally occur consecu-

tively for two or more cell generations. Such repeated crossovers are thought responsible for the occasional triple spots as well as for certain twin spots. A filling out of these gaps in our knowledge although desirable is perhaps not likely to lead to results of general significance. But this might be different with respect to other problems raised in the course of study. To name some of these: what is the "physiological Minute condition" which is responsible for somatic crossing over? Why is there correlation between the locus of Minutes and the region of crossing over induction, between sex-linked Minutes and crossing over in the X chromosome, between autosomal Minutes and crossing over in the autosome, between locus of Minute to the left or to the right of the fibre point and crossing over in the corresponding arm? What is the mechanism of the Y chromosome effect on crossing over? What is the explanation for the exceptional, but self-consistent experiment with $y\ bb^{Df}/sn^{3}$; Y females?

The mechanism of mosaic formation rests *primarily* on the occurrence of somatic crossing over. If the mechanism of fibre point separation in mitosis remains undisturbed, as has been shown to be true, then genic segregation is a *necessary consequence* of crossing over. There is thus no need to assume two separate processes (1) somatic crossing over and (2) somatic segregation.

Abnormalities in chromosome behavior as causes of mosaic formation have been recognized before. Elimination of chromosomes or non-disjunction has been the most general explanation, for example, in gynandromorphs of Drosophila (MORGAN and BRIDGES 1919), or in certain types of endosperm mosaics in corn (EMERSON 1921, 1924). The idea of possible somatic crossing over was introduced by SEREBROVSKY (1925) in an interesting paper on the appearance of aberrant feathers on chickens of different genetic constitutions. SEREBROVSKY sums up his findings by stating "crossing over cannot be either proved, or denied" and he did not recognize it as the *cause* of somatic segregation. A different interpretation of his cases was consequently given by P. HERTWIG and RITTERSHAUS (1929) and by the present author (1928a, 1933). But it must be said now that SEREBROVSKY probably came nearer to the right explanation than his critics, although certain difficulties remain.

Actual segregation of the chromosomes among somatic cells was genetically demonstrated first by PATTERSON (1929a, b; see also FRIESEN 1935) who subjected Drosophila larvae to X-rays and obtained twin spots, besides many single spots. The author "cannot state definitely the nature of the mechanism which produces the segregation. It almost certainly involves some form of synapsis . . . in the somatic cells." On the basis of our present knowledge we believe the mechanism to have been somatic crossing over as suggested earlier by PAINTER (1934). And we are

inclined to regard most cases of mosaic formation in X-rayed larvae not as results of elimination of parts of chromosomes as assumed before (PATTERSON 1930) but as indication that X-rays can induce crossing over in somatic cells.

There are some cytological findings which bear on this discussion. MCCLINTOCK (1932) obtained cytological evidence of different chromosome constitution in diverse cells of a variegated corn plant. Her finding of different degrees of increase or decrease in length of a ring-shaped chromosome in different cells pointed strongly to some kind of somatic crossing over.

In *Drosophila melanogaster* KAUFMANN (1934) could show the occasional presence of chiasmatype-like configurations between homologous chromosomes in somatic cells, before genetic evidence for somatic crossing over was available. And PETO has recently shown (1935) that chiasmata are formed in cells of root tips of *Hordeum vulgare* under the influence of radiation, a cytological correlate to PATTERSON'S experiments.

How frequently mosaic production is to be regarded as caused by somatic crossing over can be determined by new experiments only. In *Drosophila melanogaster* it seems by far the most prevalent mechanism. In all our experiments in which products of elimination or non-disjunction could be distinguished from those of somatic crossing over and segregation the majority of spots was understandable only by assuming the latter process, whereas the small minority could be explained on either hypothesis. This was true both for the smaller spots and for the occasional larger spots comprising more than one imaginal disk. There is one exception to this statement: the gynandromorphs described by MORGAN and BRIDGES (1919) and other workers cannot be explained by crossing over and segregation. This is perhaps significant. The somatic pairing of homologous chromosomes which is typical in Diptera becomes visible first in the prophase of the second cleavage division (HUETTNER 1924). As most gynandromorphic conditions originate during the first division a causal connection becomes probable.

One might suspect that the somatic chromosome pairing in Drosophila would facilitate the occurrence of crossing over. This would point to crossing over as a cause of mosaic formation in Diptera mainly. But as we do not know the reason why certain cells do undergo crossing over we are hardly justified at present in drawing this conclusion. On the other hand we should hold open the possibility of non-homologous somatic crossing over as it seems to occur in corn (MCCLINTOCK 1932, JONES 1936). In Drosophila no evidence for non-homologous crossing over is available.

Many cases of inherited mosaic formation have been described as consequences of mutations of "unstable" genes (DEMEREC 1935). The present

writer (1935) has proposed a hypothesis according to which the behavior of unstable loci should be regarded not as an internal change in a gene but as a result of "mechanical" changes at the locus, brought about by somatic crossing over. A model for such unstable loci was elaborated. Recently SCHULTZ (1936) has proposed a different cytological configuration in cases of unstable loci, which, being based on direct observation, is superior to the original speculation although probably not final either. An essential part of SCHULTZ'S scheme is again the assumption that somatic crossing over changes the cytological configuration and thus leads to mosaics.

The finding of somatic crossing over throws some light on the mechanism of chromosome separation in mitosis. Normally the passing to different poles of chromosome halves implies a separation of whole sister strands. In cases of crossing over separation may occur in the normal way for one particular point only while the rest of two sister chromosomes may pass to the same pole. This particular point is the fibre attachment locus. We have here a genetic demonstration of its role in mitosis.

That the occurrence of crossing over without reduction of chromosomes is of significance for theories of meiosis and mitosis needs hardly to be pointed out.

The value of somatic segregation as a tool for the analysis of gene action is obvious. The process has been successfully used by DEMEREC (1934 and later) in his studies of the action of small deficiencies in hypodermal segregates (cf. also STERN 1935). A few more facts have come to light in the foregoing pages: viability of hypodermal areas containing one X chromosome and two Theta duplications; or one X chromosome and one long X duplication of varying length; or two X chromosomes and similar duplications; or three X chromosomes containing two Minute-n loci. All these constitutions are lethal to zygotes. The genic unbalance represented by them is thus not able to sustain full ontogenetic development but is compatible with division and differentiation of cells of imaginal discs.

SUMMARY

(1) Mosaic areas on the body of *Drosophila melanogaster* appear on flies which are heterozygous for genes whose homozygous effect can be recognized in a small spot.

(2) The frequency of spots is increased by the presence of Minute factors.

(3) Spots of sex-linked characters occur with higher frequency when either sex-linked or autosomal Minutes are present, but sex-linked Minutes are more powerful than autosomal ones. Autosomal spots are more frequent in the case of presence of autosomal Minutes than of sex-linked

Minutes. Different Minutes show different degrees of ability to induce spot formation.

(4) The mechanism of mosaic formation is not based on simple elimination of chromosomes but on processes of somatic crossing over involving two strands of a four strand group. Segregation of the four strands occurs in an equational, typically mitotic mode in respect to the fibre points. It leads to homozygosis of originally heterozygous genes. No reduction of number of chromatids takes place in normal cases. These conclusions are derived from an analysis of types and frequencies of twin and single spots and of the number of X chromosomes present as judged by the secondary sexual characters of favorable spots. Interpretations based on experiments with certain combinations of genes have been verified by tests of validity in other combinations of the same genes.

(5) The increase of frequency of sex-linked spots is not directly dependent on the localized, material constitution of the chromosomes involved in somatic crossing over but rather on the general "phenotypic Minute reaction" in development.

(6) The relative frequencies of somatic crossovers in different regions of the X chromosomes are different from those of germinal crossovers. Somatic crossing over is more frequent near the fibre point. The presence of Minute-n accentuates this shift.

(7) The X chromosome duplication "Theta" frequently undergoes somatic crossing over with the X chromosome—more frequently in the homologous right than in the homologous left regions. Germinal crossing over involving Theta is very rare.

(8) Somatic crossing over involving Theta followed by equational segregation leads to twin segregates of the constitution 3X chromosomes-1X chromosome.

(9) The apparently exceptional behavior of the bobbed character, which does not become visible in spots, is understandable under the assumption that no somatic crossing over occurs to the right of the bobbed locus.

(10) Somatic crossing over involving the sex chromosome occurs in superfemales and in males.

(11) Somatic autosomal crossing over takes place in both sexes, though more frequently in females. A peculiar specificity of the Minute effect leads to crossovers in that arm of the third chromosome in which the Minute itself is located. Most crossovers are concentrated near the fibre point region.

(12) Somatic crossing over between X chromosomes heterozygous for the bb^{D1} inversion occurs within the inversion. It leads to a chromatid

which possesses no fibre point and is thus eliminated, and to a complementary chromatid with two fiber points. This chromatid becomes fragmented and each fragment is included in a daughter nucleus.

(13) When Theta is present in cells heterozygous for the bb^{Df} inversion the most frequent type of somatic crossover involves Theta and the not-inverted, not-bb^{Df} chromosome. (A discrepancy is pointed out between this interpretation and the observed facts.)

(14) The presence of an extra Y chromosome in flies discussed under (12) increases the frequency of somatic crossing over within the inversion to the right of sn^3 as well as the frequency of fragmentation of the two-fibre-point chromatid, also to the right of sn^3.

(15) In flies discussed under (13) the presence of an extra Y chromosome increases the frequency of crossovers involving Theta.

(16) An exceptional series of cultures with XXY females gave results which can be interpreted as caused by somatic crossing over between X and Y chromosomes, leading to XXX and X segregates.

(17) Somatic crossing over in flies heterozygous for a ring-shaped X chromosome leads to a two-fibre-point "tandem" chromatid. Segregation occurs preferentially so that the two non-crossover chromatids go to one pole and the tandem chromatid to the other. In the following division the tandem chromatid becomes fragmented.

(18) In different experiments certain segregated constitutions are not sufficiently viable to give rise to mosaic areas. Others, though not viable as zygotic constitutions, permit the formation of hypodermal spots.

(19) Under different genetic conditions different patterns of mosaics are formed. The proportion of small to larger spots can vary. In Minute-n flies crossovers to the left of *Mn* occur later in development than crossovers to the right. Various genetic constitutions have differential effects on frequency and size of spots in various body regions. Different types of spots in flies of the same constitution are differently distributed over the head, thorax and abdomen or over the different abdominal segments.

(20) In the discussion, a short survey is given with reference to mosaic formation in general and its relation to somatic segregation.

LITERATURE CITED

BEADLE, G. W. and STURTEVANT, A. H., 1935 X Chromosome inversions and meiosis in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **21**: 384–390.

BRIDGES, CALVIN B., 1925 Elimination of chromosomes due to a mutant (Minute-n) in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **11**: 701–706.

BURKART, ARTURO and STERN, CURT, 1933 Untersuchungen über eine spontane Chromosomenverlagerung bei *Drosophila melanogaster*. **64**: 310–325. Z. i. A. V.

DEMEREC, M., 1934 Biological action of small deficiencies of X chromosome of *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **20**: 354–359.

1935 Unstable genes. Bot. Reviews **1**: 233–248.

DOBZHANSKY, TH., 1934 Studies on chromosome conjugation. III. Behavior of duplicating fragments. Z. i. A. V. **68:** 134–162.

DOBZHANSKY, TH. and SCHULTZ, JACK, 1934 The distribution of sex-factors in the X chromosome of *Drosophila melanogaster*. J. Genet. **28:** 349–386.

EMERSON, R. A., 1921 Genetic evidence for aberrant chromosome behavior in maize endosperm Amer. J. Bot. **8:** 411–424.

1924 Aberrant endosperm development as a means of distinguishing linkage groups in maize. Amer. Nat. **58:** 272–277.

FRIESEN, HEINRICH, 1934 Künstliche Auslösung von Crossing-over bei Drosophila-Männchen. Biol. Zbl., **54:** 65–75.

1935 Somatische Deletionen des X-Chromosoms von *Drosophila melanogaster*. Z.i.A.V. **68:** 436–442.

GERSHENSON, S., 1933 Studies on the genetically inert region of the X-chromosome of Drosophila. I. Behavior of an X-chromosome deficient for a part of its inert region. J. Genet. **28:** 297–313.

HÅKANSSON, A., 1936 Die Reduktionsteilung in einigen Artbastarden von Pisum. Hereditas **21:** 215–222.

HERTWIG, P. and RITTERSHAUS, T., 1929 Über Fehlfedern bei gesperberten Hühnern. Arch. f. Geflügelkunde **3:** 65–76.

HUETTNER, A. F., 1924 Maturation and fertilization in *Drosophila melanogaster*. J. Morph. and Physiol. **39:** 249–265.

JONES, D. F., 1936 Segregation of color and growth-regulating genes in somatic tissue of maize. Proc. Nat. Acad. Sci. **22:** 163–166.

KAUFMANN, B. P., 1934 Somatic mitoses of *Drosophila melanogaster*. J. Morph. **56:** 125–155.

MATHER, K., 1935 Chromosome behaviour in a triploid wheat hybrid. Z. Zellf. mik. Anat. **23:** 117–138.

MORGAN, LILIAN V., 1926 Correlation between shape and behavior of a chromosome. Proc. Nat. Acad. Sci. **12:** 180–181.

1933 A closed X chromosome in *Drosophila melanogaster*. Genetics **18:** 250–283.

MORGAN, T. H. and BRIDGES, C. B., 1919 The origin of gynandromorphs. Pub. Carnegie Instn. **278:** 1–122.

MORGAN, T. H., BRIDGES, C. B. and STURTEVANT, A. H., 1925 Genetics of Drosophila. Bibilographia Genetica **2:** 1–262.

MORGAN, T. H., STURTEVANT, A. H. and BRIDGES, C. B., 1929 The constitution of the germinal material in relation to heredity. Yearb. Carnegie Instn. **28:** 1928–29, pp. 338–345.

MCCLINTOCK, BARBARA, 1932 A correlation of ring-shaped chromosomes with variegation in *Zea mays*. Proc. Nat. Acad. Sci. **18:** 677–681.

1933 The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. Z. Zellf. mik. Anat., **19:** 191–237.

MUNTZING, ARNE, 1934 Chromosome fragmentation in a Crepis hybrid. Hereditas **19:** 284–302.

OFFERMANN, C. A., 1936 Branched chromosomes as symmetrical duplications. J. Genet. **32:** 103–116.

PAINTER, T. S., 1934 A new method for the study of chromosome aberrations and the plotting of chromosome maps in *Drosophila melanogaster*. Genetics **19:** 175–188.

PATTERSON, J. T., 1929a The production of mutations in somatic cells of *Drosophila melanogaster* by means of x-rays. J. Exp. Zool. **53:** 327–372.

1929b Somatic segregation produced by x-rays in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **16:** 109–111.

1930 Proof that the entire chromosome is not eliminated in the production of somatic variations by x-rays in Drosophila. Genetics **15:** 141–149.

1932 A gene for viability in the X chromosome of Drosophila. Z.i.A.V. **60:** 125–136.

PATTERSON, J. T. and SUCHE, M. L., 1934 Crossing over induced by x-rays in Drosophila males. Genetics **19:** 223–236.

PATTERSON, J. T., STONE WILSON, and BEDICHEK, SARAH, 1935 The genetics of X-hyperploid females. Genetics **20**: 259–279.

PETO, F. H., 1935 Associations of somatic chromosomes induced by heat and chloral hydrate treatments. Canadian J. Res. **13**: 301–314.

SCHULTZ, JACK, 1929 The Minute reaction in the development of *Drosophila melanogaster*. Genetics **14**: 366–419.

1936 Variegation in Drosophila and the inert chromosome regions. Proc. Nat. Acad. Sci. **22**: 27–33.

SEREBROVSKY, A. S., 1925 "Somatic segregation" in domestic fowl. J. Genet. **16**: 33–42.

SHULL, A. and WHITTINGHILL, M., 1934 Crossovers in male *Drosophila melanogaster* induced by heat. Science **80**: 103–104.

SIVERTZEV-DOBZHANSKY, N. P. and DOBZHANSKY, TH., 1933 Deficiency and duplications for the gene bobbed in *Drosophila melanogaster*. Genetics **18**: 173–192.

SMITH, S. G., 1935 Chromosome fragmentation produced by crossing over in *Trillium erectum* L. J. Genet. **30**: 227–232.

STERN, CURT, 1927a Ein genetischer und zytologischer Beweis für Vererbung im Y-Chromosom von *Drosophila melanogaster*. Z. i. A. V. **44**: 187–231.

1927b Über Chromosomenelimination bei der Taufliege. Naturwiss. **15**: 740–746.

1928a Fortschritte der Chromosomentheorie der Vererbung. Ergebn. Biol. **4**: 205–359.

1928b Elimination von Autosomenteilen bei *Drosophila melanogaster*. Z. i. A. V. Sup. **2**: 1403–1404.

1929 Untersuchungen über Aberrationen des Y-Chromosoms von *Drosophila melanogaster*. Z. Abstgsl. **51**: 253–353.

1933 Faktorenkoppelung und Faktorenaustausch. Handb. Vererbgewiss. **1**: 1–331.

1934 Crossing-over and segregation in somatic cells of *Drosophila melanogaster* (Abstract) Amer. Nat. **68**: 164–165.

1935a Further studies on somatic crossing-over and segregation (Abstract). Amer. Nat. **69**: 81–82.

1935b The behavior of unstable genic loci-an hypothesis. Proc. Nat. Acad. Sci. **21**:202–208.

1935c The effect of yellow-scute gene deficiency on somatic cells of Drosophila. Proc. Nat. Acad. Sci. **21**: 374–379.

STERN, C. and RENTSCHLER, V., 1936 The effect of temperature on the frequency of somatic crossing over in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **22**: 451–453.

STURTEVANT, A. H., 1932 The use of mosaics in the study of the developmental effect of genes. Proc. Sixth Int. Congress Genetics **1**: 304–307.